

```

=> encapsulation
    20666 ENCAPSULATION
    116 ENCAPSULATIONS
L1    20716 ENCAPSULATION
        (ENCAPSULATION OR ENCAPSULATIONS)

=> capsid (w) protein
    8854 CAPSID
    1406 CAPSIDS
    9299 CAPSID
        (CAPSID OR CAPSIDS)
    1467113 PROTEIN
    979368 PROTEINS
    1695050 PROTEIN
        (PROTEIN OR PROTEINS)
L2    5043 CAPSID (W) PROTEIN

=> L1 and L2
L3    7 L1 AND L2

=> L2 and receptor
    496186 RECEPTOR
    453339 RECEPTORS
    590791 RECEPTOR
        (RECEPTOR OR RECEPTORS)
L4    276 L2 AND RECEPTOR

=> fusion (w) protein
    209560 FUSION
    7762 FUSIONS
    213796 FUSION
        (FUSION OR FUSIONS)
    1467113 PROTEIN
    979368 PROTEINS
    1695050 PROTEIN
        (PROTEIN OR PROTEINS)
L5    31285 FUSION (W) PROTEIN

=> L5 and L4
L6    24 L5 AND L4

=> " virus like particle"
    275407 "VIRUS"
    56290 "VIRUSES"
    285115 "VIRUS"
        ("VIRUS" OR "VIRUSES")
    548021 "LIKE"
    180 "LIKES"
    548176 "LIKE"
        ("LIKE" OR "LIKES")
    557157 "PARTICLE"
    629010 "PARTICLES"
    947448 "PARTICLE"
        ("PARTICLE" OR "PARTICLES")
L7    1523 " VIRUS LIKE PARTICLE"
        ("VIRUS" (W) "LIKE" (W) "PARTICLE")

=> L7 and L6
L8    5 L7 AND L6

```

L6 ANSWER 6 OF 24 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:338691 CAPLUS

DOCUMENT NUMBER: 134:348931

TITLE: Use of reversibly immobilized complex forming proteins

in the packaging of molecules in protein shells

INVENTOR(S): Boehm, Gerald; Esser, Dirk; Schmidt, Ulrich

PATENT ASSIGNEE(S): Acgt Progenomics A.-G., Germany

SOURCE: PCT Int. Appl., 62 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: German

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001032852	A2	20010510	WO 2000-EP10878	20001103
WO 2001032852	A3	20011213		
W:				
AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW:				
GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
DE 19952982	A1	20010517	DE 1999-19952982	19991103
EP 1228199	A2	20020807	EP 2000-971419	20001103
R:				
AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
PRIORITY APPLN. INFO.:			DE 1999-19952982 A	19991103
			WO 2000-EP10878 W	20001103

AB The invention relates to a method for packaging mol. substances in protein

shells. A component of the protein shell is immobilized on a suitable matrix. The immobilized protein is incubated with the substance of interest to allow binding of the two mols. The protein shell fragment with the bound mol. is then released from the matrix. The shell fragments

carrying the mol. is incubated with other protein shell fragments to form a protein shell, whereby the sepn. and assembly can be carried out in any order. The method can be used to encapsulate nucleic acids in viral capsids in vitro without the need for packaging cell lines. The **capsid proteins** may be further modified by inclusion of affinity ligands for cell surface **receptors**. Use of variants of polyomavirus VP1 protein to encapsulate a plasmid is demonstrated. Specifically, a **fusion protein** of VP1, an intein, and a chitin binding domain was constructed and immobilized on a chitin surface. After immobilization, the **capsid protein** could be released by treatment with dithiothreitol and hydroxylamine.

L6 ANSWER 5 OF 24 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2001:463085 CAPLUS

DOCUMENT NUMBER: 135:56904

TITLE: Adeno-associated virus major **capsid protein** fusion with nuclear localization signal for virus-like particle formation

INVENTOR(S): Handa, Hiroshi; Hooock, Meinor; Ishizu, Kenichiro; Watanabe, Hajime

PATENT ASSIGNEE(S): Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 33 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2001169777	A2	20010626	JP 1999-249140	19990730
PRIORITY APPLN. INFO.:			JP 1999-249140	19990730

AB Recombinant adeno-assocd. virus major **capsid proteins** with a nuclear localization signal peptide (NLS), and their use for virus-like particle (VLP) formation, are disclosed. NLS from SV40 virus large T antigen, SV40 virus VP1 or VP2, human lamin A, histone H2B, adenovirus E1A, polyomavirus large T antigen, human c-myc, nucleoplasmin, N1, rat glucocorticoid **receptor**, can be used. Adeno-assocd. virus capsids are composed of three proteins, VP1, VP2, and VP3.

Although

VP1 is necessary for viral infection, it is not essential for capsid formation. The other **capsid proteins**, VP2 and VP3, are sufficient for capsid formation, but the functional roles of each protein are still not well understood. By analyzing a series of deletion mutants of VP2, we identified a region necessary for nuclear transfer of VP2 and found that the efficiency of nuclear localization of the **capsid proteins** and the efficiency of virus-like particle (VLP) formation correlated well. To confirm the importance of the nuclear localization of the **capsid proteins**, we fused the nuclear localization signal of simian virus 40 large T antigen to VP3 protein. We show that this **fusion protein** could form VLP, indicating that the VP2-specific region located on the N-terminal side of the protein is not structurally required. This finding

suggests that VP3 has sufficient information for VLP formation and that VP2 is necessary only for nuclear transfer of the **capsid proteins**. VLP formation in Sf9 cells is describe

Li 09/750,185

=> d his 1

(FILE 'MEDLINE, HCAPLUS, BIOSIS, SCISEARCH, AGRICOLA, EMBASE' ENTERED AT
10:57:35 ON 21 NOV 2002)

L51 52 S L48 OR L50

=> d que l51

L1 1549 SEA HUNT N?/AU
L3 1810 SEA VLP AND VIRUS?
L4 11143 SEA VIRUS(3A) LIKE(3A) PARTICLE#
L5 952537 SEA INCORPORAT?
L6 99864 SEA ENCAPSULAT?
L7 12595 SEA HETEROLOG? (3A) (PROTEIN# OR POLYPEPTIDE#)
L8 3090288 SEA RECEPTOR?
L9 133177 SEA ION(3A) CHANNEL#
L10 3232805 SEA ENZYME#
L11 151954 SEA ADHESION(3A) (POLYPEPTIDE# OR PROTEIN# OR MOLECULE#)
L12 18736 SEA MEMBRAN? (3A) PORE#
L13 44997 SEA G(A) PROTEIN#(5A) COUPLE?(5A) RECEPTOR#
L14 4215 SEA VIRUS(3A) CAPSID(3A) (POLYPEPTIDE# OR PROTEIN#)
L16 13016 SEA GAG(3A) GENE?
L20 465876 SEA ASSEMBL?
L21 3 SEA L1 AND (L3 OR L4)
L22 4 SEA (L3 OR L4) AND L13
L23 39 SEA (L3 OR L4) AND L7
L24 17 SEA (L3 OR L4) AND (L9 OR L11 OR L12)
L25 1 SEA (L3 OR L4) AND (HETEROLOG?(3A) L8)
L26 1796 SEA (L3 OR L4) AND L20
L27 397 SEA L26 AND (L14 OR L16)
L28 41 SEA (L5 OR L6) AND L27
L30 31 SEA (L3 OR L4) AND ((CHIMER? OR EXPRESS?) (3A) ((L8 OR L9 OR L10
OR L11 OR L12)))
L31 127 SEA ((L23 OR L24 OR L25)) OR L28 OR L30
L32 11 SEA L31 AND (DIMER# OR OLIGOMER# OR MULTIMER?)
L33 116 SEA L31 NOT L32
L34 5 SEA FILE=EMBASE (L3 OR L4) AND L7
L35 3 SEA FILE=EMBASE (L3 OR L4) AND (L9 OR L11 OR L12)
L37 381 SEA FILE=EMBASE (L3 OR L4) AND L20
L38 54 SEA FILE=EMBASE L37 AND (L14 OR L16)
L39 4 SEA FILE=EMBASE L38 AND (L5 OR L6)
L40 6 SEA FILE=EMBASE (L3 OR L4) AND ((CHIMER? OR EXPRESS?) (3A) ((L8
OR L9 OR L10 OR L11 OR L12)))
L41 18 SEA FILE=EMBASE L34 OR L35 OR L39 OR L40
L42 2 SEA FILE=EMBASE L41 AND (DIMER# OR OLIGOMER# OR MULTIMER?)
L43 16 SEA FILE=EMBASE L41 NOT L42
L44 16 SEA L21 OR L22 OR L32
L45 10 DUP REM L44 L42 (8 DUPLICATES REMOVED)
L46 63 DUP REM L33 L43 (69 DUPLICATES REMOVED)
L47 71 SEA L45 OR L46
L48 2 SEA L47 AND FORCE#
L49 69 SEA L47 NOT L48
L50 50 SEA L49 NOT PY>2000
L51 52 SEA L48 OR L50

=> d ibib abs l51 1-52

L51 ANSWER 1 OF 52 MEDLINE
ACCESSION NUMBER: 2000459423 MEDLINE

DOCUMENT NUMBER: 20411443 PubMed ID: 10954570
TITLE: Membrane targeting properties of a herpesvirus tegument
protein-retrovirus Gag chimera.
AUTHOR: Bowzard J B; Visalli R J; Wilson C B; Loomis J S; Callahan
E M; Courtney R J; Wills J W
CORPORATE SOURCE: Department of Microbiology and Immunology, The Pennsylvania
State University College of Medicine, Hershey, Pennsylvania
17033, USA.
CONTRACT NUMBER: CA42460 (NCI)
CA47482 (NCI)
CA60395 (NCI)
+
SOURCE: JOURNAL OF VIROLOGY, (2000 Sep) 74 (18) 8692-9.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20001005
Last Updated on STN: 20001005
Entered Medline: 20000927

AB The retroviral Gag protein is capable of directing the production and release of **virus-like particles** in the absence of all other viral components. Budding normally occurs after Gag is transported to the plasma membrane by its membrane-targeting and -binding (M) domain. In the Rous sarcoma virus (RSV) Gag protein, the M domain is contained within the first 86 amino acids. When M is deleted, membrane association and budding fail to occur. Budding is restored when M is replaced with foreign membrane-binding sequences, such as that of the Src oncoprotein. Moreover, the RSV M domain is capable of targeting **heterologous proteins** to the plasma membrane. Although the solution structure of the RSV M domain has been determined, the mechanism by which M specifically targets Gag to the plasma membrane rather than to one or more of the large number of internal membrane surfaces (e.g., the Golgi apparatus, endoplasmic reticulum, and nuclear, mitochondrial, or lysosomal membranes) is unknown. To further investigate the requirements for targeting proteins to discrete cellular locations, we have replaced the M domain of RSV with the product of the unique long region 11 (U(L)11) gene of herpes simplex virus type 1. This 96-amino-acid myristylated protein is thought to be involved in virion transport and envelopment at internal membrane sites. When the first 100 amino acids of RSV Gag (including the M domain) were replaced by the entire UL11 sequence, the chimeric protein localized at and budded into the Golgi apparatus rather than being targeted to the plasma membrane. Myristate was found to be required for this specific targeting, as were the first 49 amino acids of UL11, which contain an acidic cluster motif. In addition to shedding new light on UL11, these experiments demonstrate that RSV Gag can be directed to internal cellular membranes and suggest that regions outside of the M domain do not contain a dominant plasma membrane-targeting motif.

L51 ANSWER 2 OF 52 MEDLINE
ACCESSION NUMBER: 2000283795 MEDLINE
DOCUMENT NUMBER: 20283795 PubMed ID: 10823843
TITLE: Efficient particle production by minimal Gag constructs which retain the carboxy-terminal domain of human immunodeficiency **virus** type 1 capsid-p2 and a late **assembly** domain.

AUTHOR: Accola M A; Strack B; Gottlinger H G
CORPORATE SOURCE: Department of Cancer Immunology and AIDS, Dana-Farber
Cancer Institute, Harvard Medical School, Boston,
Massachusetts 02115, USA.

CONTRACT NUMBER: AI28691 (NIAID)

AI29873 (NIAID)

SOURCE: JOURNAL OF VIROLOGY, (2000 Jun) 74 (12) 5395-402.

Journal code: 0113724. ISSN: 0022-538X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 200006

ENTRY DATE: Entered STN: 20000706

Last Updated on STN: 20000706

Entered Medline: 20000629

AB The human immunodeficiency virus type 1 (HIV-1) Gag precursor Pr55(gag) by itself is capable of **assembling** into retrovirus-like particles (**VLP**). In the present study, we attempted to identify the minimal Gag sequences required for the formation of **VLP**. Our results show that about 80% of Pr55(gag) can be either deleted or replaced by heterologous sequences without significantly compromising **VLP** production. The smallest chimeric molecule still able to efficiently form **VLP** was only about 16 kDa. This minimal Gag construct contained the leucine zipper domain of the yeast transcription factor GCN4 to substitute for the **assembly** function of nucleocapsid (NC), followed by a P-P-P-P-Y motif to provide late budding (L) domain function, and retained only the myristylation signal and the C-terminal capsid-p2 domain of Pr55(gag). We also show that the L domain function of HIV-1 p6(gag) is not dependent on the presence of an active viral protease and that the NC domain of Pr55(gag) is dispensable for the **incorporation** of Vpr into **VLP**.

L51 ANSWER 3 OF 52 MEDLINE

ACCESSION NUMBER: 2000173735 MEDLINE

DOCUMENT NUMBER: 20173735 PubMed ID: 10708461

TITLE: **Assembly** and processing of human immunodeficiency virus Gag mutants containing a partial replacement of the matrix domain by the viral protease domain.

AUTHOR: Wang C T; Chou Y C; Chiang C C

CORPORATE SOURCE: Institute of Clinical Medicine, National Yang-Ming
University School of Medicine, and Department of Medical
Research and Education, Taipei Veterans General Hospital,
Taipei 112, Taiwan, Republic of China..
ctwang@vghtpe.gov.tw

SOURCE: JOURNAL OF VIROLOGY, (2000 Apr) 74 (7) 3418-22.

Journal code: 0113724. ISSN: 0022-538X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 200004

ENTRY DATE: Entered STN: 20000413

Last Updated on STN: 20000413

Entered Medline: 20000407

AB We constructed human immunodeficiency virus (HIV) mutants by replacing the matrix domain with sequences encoding the viral protease or p6* and protease. The chimeras retaining matrix myristylation and processing signals underwent efficient autoprocessing with severely defective

particle budding. The budding defects of the chimeras were rescued by suppressing the chimera protease activity either through addition of an HIV protease inhibitor or through inactivating the chimera protease via a substitution mutation of the catalytic aspartic acid residue. This resulted in the release of chimeric **virus-like particles** with the density of a wild-type retrovirus particle. In addition, the **assembly**-competent but processing-defective chimeras produced proteolytically processed particles with significant reverse transcriptase activity when a downstream native pol gene was present. These results suggest that HIV has the potential to adapt heterologous sequences in place of the matrix sequence without major effects on **virus-like particle** budding. In addition, the positions of the protease and substrate accessibility may contribute significantly toward avoiding a premature Gag or Gag-Pol process, which leads to severe defects in both particle budding and **incorporation**.

L51 ANSWER 4 OF 52 MEDLINE
 ACCESSION NUMBER: 2000173445 MEDLINE
 DOCUMENT NUMBER: 20173445 PubMed ID: 10710211
 TITLE: Production and characterization of simian--human immunodeficiency **virus-like particles**.
 AUTHOR: Yao Q; Kuhlmann F M; Eller R; Compans R W; Chen C
 CORPORATE SOURCE: Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia 30322, USA.. qyao@bimcore.emory.edu
 CONTRACT NUMBER: AI 42691 (NIAID)
 AI 43068 (NIAID)
 HL 61943 (NHLBI)
 +
 SOURCE: AIDS RESEARCH AND HUMAN RETROVIRUSES, (2000 Feb 10) 16 (3) 227-36.
 Journal code: 8709376. ISSN: 0889-2229.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 200004
 ENTRY DATE: Entered STN: 20000427
 Last Updated on STN: 20000427
 Entered Medline: 20000420

AB We have produced and characterized, in a baculovirus expression system, simian-human immunodeficiency **virus-like particles** (SHIV VLPs) containing SIV Gag and HIV envelope (Env) proteins. Recombinant SIV gag (SIVmac239) and full-length or cytoplasmic domain-truncated HIV env from either HIV BH10 or HIV 89.6 virus were coexpressed in insect cells and Env **incorporation** into released SHIV VLPs was characterized. The expression level of the Env protein was found to be about 20-50% higher in both strains producing the truncated Env. Cell surface expression of the truncated Env proteins was found to be about eightfold higher than that of the full-length Env proteins. Furthermore, the truncated Env proteins exhibited higher levels of cleavage into gp120 and gp41 compared with the full-length Env. The SHIV VLPs produced by the coexpression of SIV gag and truncated HIV env contained both precursor (gp160) and gp120, while predominantly gp160 was found in the VLPs containing full-length Env. Coinfection of a recombinant virus expressing the protease furin also resulted in more efficient cleavage of gp160 to gp120. Both full-length and truncated Env were found

to induce CD4+ cell fusion. Analysis of VLPs by immunoelectron microscopy demonstrated the **incorporation** of both full-length and truncated Env on the surface of VLPs. Truncated Env also was **incorporated** at higher levels on the surfaces of VLPs than full-length Env. The **assembly** of VLPs containing biologically active Env proteins may be useful in vaccine development and in functional studies of the HIV envelope protein.

L51 ANSWER 5 OF 52 MEDLINE
 ACCESSION NUMBER: 1999447458 MEDLINE
 DOCUMENT NUMBER: 99447458 PubMed ID: 10516586
 TITLE: Optimized production of recombinant bluetongue core-like particles produced by the baculovirus expression system.
 AUTHOR: Zheng Y Z; Greenfield P F; Reid S
 CORPORATE SOURCE: Department of Chemical Engineering, The University of Queensland, QLD, 4072, Australia.. yuanZ@qimr.edu.au
 SOURCE: BIOTECHNOLOGY AND BIOENGINEERING, (1999 Dec 5) 65 (5) 600-4.
 Journal code: 7502021. ISSN: 0006-3592.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199911
 ENTRY DATE: Entered STN: 20000113
 Last Updated on STN: 20000113
 Entered Medline: 19991130

AB The baculovirus-expression vector system (BEVS) has been widely used for the experimental production of many human and animal single- and multi-unit vaccines, **heterologous proteins**, and viral insecticides. In this work, the production of recombinant bluetongue **virus core-like particles** (CLPs); using Sf9 cells in shaker-suspension culture with the SF900 II medium (GIBCO, NY), has been studied. This system involved the simultaneous production of two proteins, VP7 and VP3, and was shown to achieve high volumetric productivities. The key parameters of the time of infection (TOI), and the multiplicity of infection (MOI) were studied. The results show that the peak-volumetric yields and cell-specific yields achieved using low MOIs at low-cell densities were the same as those obtained following infections with a high MOI at high-cell densities. This work establishes the feasibility of using low MOIs in the baculovirus system to produce complex multiprotein particles.
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L51 ANSWER 6 OF 52 MEDLINE
 ACCESSION NUMBER: 1999389795 MEDLINE
 DOCUMENT NUMBER: 99389795 PubMed ID: 10459153
 TITLE: Sequence requirements for **incorporation** of human immunodeficiency virus gag-beta-galactosidase fusion proteins into **virus-like particles**.
 AUTHOR: Wang C T; Lai H Y; Yang C C
 CORPORATE SOURCE: Institute of Clinical Medicine, National Yang-Ming University School of Medicine, and Department of Medical Research and Education, Veterans General Hospital-Taipei, Taiwan, Republic of China.. ctwang@vghtpe.gov.tw
 SOURCE: JOURNAL OF MEDICAL VIROLOGY, (1999 Oct) 59 (2) 180-8.
 Journal code: 7705876. ISSN: 0146-6615.
 PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199910
 ENTRY DATE: Entered STN: 19991026
 Last Updated on STN: 19991026
 Entered Medline: 19991014

AB The **incorporation** of human immunodeficiency virus type 1 (HIV-1) Gag-beta-galactosidase (Gag-beta-gal; GBG) fusion proteins into HIV **virus-like particles** in the presence of HIV Gag proteins was studied. HIV Gag-beta-gal fusion constructs were cotransfected individually into COS7 cells with or without an HIV Gag protein expression plasmid. Release of HIV GBG fusion proteins from the cells were measured by assay of the medium versus intracellular beta-gal activities. Analysis indicates that fusion proteins (constructs HIVGBG, GBG 1919 and 1877) retaining the C-terminal portion of the CA and the adjacent NC domains were efficiently **assembled** into **virus-like particles**. Fusion proteins with deleted sequences covering the N-terminal portions of the gag sequences (GBG 831, 1147, 1419, 1447, 1511, 1552, 1600, 1630, 1684, 1715, and 1752) were impaired in entry into **virus-like particles**. The presence of CA major homology region (MHR) in the fusion proteins had no significant effects on inducing fusion protein **incorporation** when the C-terminal CA sequences in the fusion proteins were truncated (GBG 1841 and 1801). Subcellular fractionation studies indicated that most fusion proteins including the nonmyristylated one were enriched in the crude membrane fraction. Exceptions to this rule were fusion proteins with intact MHR but truncated C-terminal CA sequences, which possessed low levels of membrane association. However, **assembly** of fusion proteins into HIV Gag particles did not correlate with their subcellular fractionation or immunofluorescence localization patterns. Overall, the studies suggest that the very C-terminal CA and adjacent NC sequences are the primary determinants for **incorporation** of HIV Gag-beta-gal fusion proteins into virus particles.
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L51 ANSWER 7 OF 52 MEDLINE
 ACCESSION NUMBER: 1998440533 MEDLINE
 DOCUMENT NUMBER: 98440533 PubMed ID: 9765414
 TITLE: Proteolytic processing and assembly of gag and gag-pol proteins of TED, a baculovirus-associated retrotransposon of the gypsy family.
 AUTHOR: Hajek K L; Friesen P D
 CORPORATE SOURCE: Graduate Program in Cellular and Molecular Biology, Graduate School and College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706, USA.
 CONTRACT NUMBER: AI25557 (NIAID)
 GM07215 (NIGMS)
 SOURCE: JOURNAL OF VIROLOGY, (1998 Nov) 72 (11) 8718-24.
 Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199811
 ENTRY DATE: Entered STN: 19990106
 Last Updated on STN: 19990106

Entered Medline: 19981105

AB TED (transposable element D) is an env-containing member of the gypsy family of retrotransposons that represents a possible retrovirus of invertebrates. This lepidopteran (moth) retroelement contains gag and pol genes that encode proteins capable of forming **viruslike** particles (**VLP**) with reverse transcriptase. Since **VLP** are likely intermediates in TED transposition, we investigated the roles of gag and pol in TED capsid assembly and maturation. By using constructed baculovirus vectors and TED Gag-specific antiserum, we show that the principal translation product of gag (Pr55(gag)) is cleaved to produce a single **VLP** structural protein, p37(gag). Replacement of Asp436 within the retrovirus-like active site of the pol-encoded protease (PR) abolished Pr55(gag) cleavage and demonstrated the requirement for PR in capsid processing. As shown by expression of an in-frame fusion of TED gag and pol, PR is derived from the Gag-Pol polyprotein Pr195(gag-pol). The PR cleavage site within Pr55(gag) was mapped to a position near the junction of a basic, nucleocapsid-like domain and a C-terminal acidic domain. Once released by cleavage, the C-terminal fragment was not detected. This acidic fragment was dispensable for **VLP** assembly, as demonstrated by the formation of **VLP** by C-terminal Pr55(gag) truncation proteins and replacement of the acidic domain with a **heterologous protein**. In contrast, C-terminal deletions that extended into the adjacent nucleocapsid-like domain of Pr55(gag) abolished **VLP** recovery and demonstrated that this central region contributes to **VLP** assembly or stability, or both. Collectively, these data suggest that the single TED protein p37(gag) provides both capsid and nucleocapsid functions. TED may therefore use a simple processing strategy for **VLP** assembly and genome packaging.

L51 ANSWER 8 OF 52 MEDLINE

ACCESSION NUMBER: 1998166861 MEDLINE

DOCUMENT NUMBER: 98166861 PubMed ID: 9505962

TITLE: Characterization of an integrase mutant of feline immunodeficiency virus.

AUTHOR: Tomonaga K; Itagaki S I; Kashiwase H; Kawaguchi Y; Inoshima Y; Ikeda Y; Mikami T

CORPORATE SOURCE: Department of Veterinary Microbiology, University of Tokyo, Japan.

SOURCE: ARCHIVES OF VIROLOGY, (1998) 143 (1) 1-14.
Journal code: 7506870. ISSN: 0304-8608.

PUB. COUNTRY: Austria

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 199804

ENTRY DATE: Entered STN: 19980410

Last Updated on STN: 19980410

Entered Medline: 19980402

AB The role of the integrase region of feline immunodeficiency virus (FIV) in viral replication was examined using an integrase mutant clone of FIV which carries a frameshift mutation in the region. Upon transfection, although the integrase mutant was able to release **virus-like particles** into the supernatant from the transfected cells, the virions produced by the mutant contained unprocessed gag precursor protein and undetectable levels of reverse transcriptase activity. Furthermore, the mutant virions were unable to direct the synthesis of viral DNA after infection in target cells. To understand this phenotype of the integrase mutant in more detail, we constructed a gag-pol expression plasmid from an FIV molecular clone and assayed roles of the

integrase region on virus particle formation following transfection. When an inframe deletion was introduced into the protease region of the expression plasmid, the mutant was able to efficiently release gag- and gag-pol precursor proteins into the supernatant from the transfected cells. An expression plasmid with mutations in both the protease and integrase regions, however, failed to release the gag-pol precursor protein from the cells. These results suggested an essential role for the integrase region for efficient **incorporation** of the gag-pol precursor into the virions.

L51 ANSWER 9 OF 52 MEDLINE
 ACCESSION NUMBER: 97145453 MEDLINE
 DOCUMENT NUMBER: 97145453 PubMed ID: 8991095
 TITLE: Targeting of Moloney murine leukemia virus gag precursor to the site of virus budding.
 AUTHOR: Suomalainen M; Hultenby K; Garoff H
 CORPORATE SOURCE: Department of Bioscience at Novum, Sweden.
 SOURCE: JOURNAL OF CELL BIOLOGY, (1996 Dec) 135 (6 Pt 2) 1841-52.
 Journal code: 0375356. ISSN: 0021-9525.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199702
 ENTRY DATE: Entered STN: 19970219
 Last Updated on STN: 20000303
 Entered Medline: 19970204

AB Retrovirus Moloney murine leukemia virus (M-MuLV) matures by budding at the cell surface. Central to the budding process is the myristoylated viral core protein precursor Gag which, even in the absence of all other viral components, is capable of associating with the cytoplasmic leaflet of the plasma membrane and **assembling** into extracellular **virus-like particles**. In this paper we have used heterologous, Semliki Forest virus-driven, expression of M-MuLV Gag to study the mechanism by which this protein is targeted to the cell surface. In pulse-chase experiments, BFA, monensin, and 20 degrees C block did not affect **incorporation** of Gag into extracellular particles thereby indicating that the secretory pathway is not involved in targeting of Gag to the cell surface. Subcellular fractionation studies demonstrated that newly synthesized Gag became rapidly and efficiently associated with membranes which had a density similar to that of plasma membrane-derived vesicles. Protease-protection studies confirmed that the Gag-containing membranes were of plasma membrane origin, since in crude cell homogenates, the bulk of newly synthesized Gag was protease-resistant as expected of a protein that binds to the cytoplasmic leaflet of the plasma membrane. Taken together these data indicate that targeting of M-MuLV Gag to the cell surface proceeds via direct insertion of the protein to the cytoplasmic side of the plasma membrane. Furthermore, since the membrane insertion reaction is highly efficient and specific, this suggests that the reaction is dependent on as-yet-unidentified cellular factors.

L51 ANSWER 10 OF 52 MEDLINE
 ACCESSION NUMBER: 97143416 MEDLINE
 DOCUMENT NUMBER: 97143416 PubMed ID: 8989427
 TITLE: Characterization of the expression and immunogenicity of poliovirus replicons that encode simian immunodeficiency virus SIVmac239 Gag or envelope SU proteins.
 AUTHOR: Anderson M J; Porter D C; Moldoveanu Z; Fletcher T M 3rd; McPherson S; Morrow C D

CORPORATE SOURCE: Department of Microbiology, University of Alabama at
Birmingham 35294, USA.
CONTRACT NUMBER: AI27767 (NIAID)
AI28147 (NIAID)
T32-AI 07150 (NIAID)
+
SOURCE: AIDS RESEARCH AND HUMAN RETROVIRUSES, (1997 Jan 1) 13 (1)
53-62.
Journal code: 8709376. ISSN: 0889-2229.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 19970327
Last Updated on STN: 19970327
Entered Medline: 19970319

AB The effectiveness of the poliovirus vaccines to induce both systemic and mucosal immunity has prompted the development of this virus as a vector in which to express foreign proteins. Our laboratory has previously reported on the construction and characterization of poliovirus genomes that encode HIV-1 proteins (Porter DC, et al.: J Virol 1996;70:2643-2649). To develop this system further, we have constructed poliovirus genomes, referred to as replicons, which encode the SIVmac239 Gag or Env SU in place of the poliovirus capsid gene (P1). Since the replicons do not encode capsid proteins, they are encapsidated into poliovirus by passage with a recombinant vaccinia virus, VVP1, which provides the poliovirus capsid proteins in trans. Using this system, we have derived stocks of the encapsidated replicons which encode the SIVmac239 or Env SU protein. Infection of cells with the replicon that encodes SIVmac239 Gag resulted in the expression of a 55-kDa protein that was released from the infected cells. Analysis of the sedimentation of the released proteins by sucrose density gradient centrifugation revealed that the protein was released from the cell in the form of a **virus-like particle**. Infection of cells with the replicons encoding the SIVmac239 Env SU resulted in the expression of a 63-kDa protein, corresponding to the molecular mass predicted for the nonglycosylated SIVmac239 SU protein. A second protein with a molecular mass greater than 160 kDa was also immunoprecipitated. After enzymatic deglycosylation, this protein migrated at a molecular mass consistent with that for an Env SU **dimer**. Analysis of the medium from cells infected with the replicon encoding SIVmac239 Env SU revealed the presence of a protein of molecular mass 85-90 kDa, possibly representing a fragment of the SIVmac239 or Env SU protein. To determine the immunogenicity of the replicons encoding SIVmac239 Gag or Env SU, transgenic mice that **express** the human **receptor** for poliovirus, and are thus susceptible to poliovirus, were immunized via the intramuscular route. A serum antibody response to SIV envelope was detected following booster immunization, establishing that the encapsidated replicon was immunogenic. Finally, we demonstrate that the replicons have the capacity to infect peripheral blood mononuclear monocytes/macrophages, suggesting that this cell is a possible target for in vivo infection. The results of our studies, then, lend further support for the development and application of recombinant poliovirus replicons in a vaccine strategy.

L51 ANSWER 11 OF 52 MEDLINE
ACCESSION NUMBER: 96323161 MEDLINE
DOCUMENT NUMBER: 96323161 PubMed ID: 8709267
TITLE: Identification of domains in the simian immunodeficiency

virus matrix protein essential for **assembly** and envelope glycoprotein **incorporation**.
 AUTHOR: Gonzalez S A; Burny A; Affranchino J L
 CORPORATE SOURCE: Centro de Virologia Animal, Buenos Aires, Argentina.. sag@cevan.sld.ar
 SOURCE: JOURNAL OF VIROLOGY, (1996 Sep) 70 (9) 6384-9. Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199609
 ENTRY DATE: Entered STN: 19960919
 Last Updated on STN: 19970203
 Entered Medline: 19960910

AB The matrix domain (MA) of the simian immunodeficiency virus (SIV) is encoded by the amino-terminal region of the Gag polyprotein precursor and is the component of the viral capsid that lines the inner surface of the virus envelope. To define domains of the SIV MA protein that are involved in virus morphogenesis, deletion and substitution mutations were introduced in this protein in the context of a gag-protease construct and expressed in the vaccinia virus vector system. The MA mutants were characterized with respect to synthesis and processing of the Gag precursor, **assembly** and release of **virus-like particles**, and **incorporation** of the envelope (Env) glycoprotein into particles. We have identified two regions of the SIV MA which are critical for particle formation. Both domains are located in a central hydrophobic alpha-helix of the SIV MA, according to data on the structure of this protein. In addition, we have characterized a domain whose mutation impairs the **incorporation** of SIV Env glycoproteins with long transmembrane cytoplasmic tails into particles. Interestingly, these mutant particles retained the ability to associate with SIV Env proteins with short cytoplasmic tails.

L51 ANSWER 12 OF 52 MEDLINE
 ACCESSION NUMBER: 96323101 MEDLINE
 DOCUMENT NUMBER: 96323101 PubMed ID: 8709207
 TITLE: In vitro generation and type-specific neutralization of a human papillomavirus type 16 virion pseudotype.
 AUTHOR: Roden R B; Greenstone H L; Kirnbauer R; Booy F P; Jessie J; Lowy D R; Schiller J T
 CORPORATE SOURCE: Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD 20892, USA.
 SOURCE: JOURNAL OF VIROLOGY, (1996 Sep) 70 (9) 5875-83. Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199609
 ENTRY DATE: Entered STN: 19960919
 Last Updated on STN: 19970203
 Entered Medline: 19960910

AB We report a system for generating infectious papillomaviruses in vitro that facilitates the analysis of papillomavirus assembly, infectivity, and serologic relatedness. Cultured hamster BPHE-1 cells harboring autonomously replicating bovine papillomavirus type 1 (BPV1) genomes were infected with recombinant Semliki Forest viruses that express the structural proteins of BPV1. When plated on C127 cells, extracts from

cells expressing L1 and L2 together induced numerous transformed foci that could be specifically prevented by BPV neutralizing antibodies, demonstrating that BPV infection was responsible for the focal transformation. Extracts from BPHE-1 cells expressing L1 or L2 separately were not infectious. Although Semliki Forest virus-expressed L1 self-assembled into **virus-like particles** (VLPs), viral DNA was detected in particles only when L2 was coexpressed with L1, indicating that genome encapsidation requires L2. Expression of human papillomavirus type 16 (HPV16) L1 and L2 together in BPHE-1 cells also yielded infectious virus. These pseudotyped virions were neutralized by antiserum to HPV16 VLPs derived from European (114/K) or African (Z-1194) HPV16 variants but not by antisera to BPV VLPs, to a poorly assembling mutant HPV16 L1 protein, or to VLPs of closely related genital HPV types. Extracts from BPHE-1 cells coexpressing BPV L1 and HPV16 L2 or HPV16 L1 and BPV L2 were not infectious. We conclude that (i) mouse C127 cells **express** the cell surface **receptor** for HPV16 and are able to uncoat HPV16 capsids; (ii) if a papillomavirus DNA packaging signal exists, then it is conserved between the BPV and HPV16 genomes; (iii) functional L1-L2 interaction exhibits type specificity; and (iv) protection by HPV **virus-like particle** vaccines is likely to be type specific.

L51 ANSWER 13 OF 52 MEDLINE

ACCESSION NUMBER: 96266412 MEDLINE

DOCUMENT NUMBER: 96266412 PubMed ID: 8661411

TITLE: **Assembly** and release of SIV env proteins with full-length or truncated cytoplasmic domains.

AUTHOR: Vzorov A N; Compans R W

CORPORATE SOURCE: Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia 30322, USA.

CONTRACT NUMBER: AI 28147 (NIAID)

AI 34242 (NIAID)

AI 38501 (NIAID)

+

SOURCE: VIROLOGY, (1996 Jul 1) 221 (1) 22-33.
Journal code: 0110674. ISSN: 0042-6822.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 199608

ENTRY DATE: Entered STN: 19960822

Last Updated on STN: 19970203

Entered Medline: 19960814

AB We have used recombinant vaccinia viruses expressing full-length or truncated **gag** or env **genes** of SIVmac239 to investigate the requirements for **assembly** of SIV proteins. We observed that **assembly of virus-like particles** (VLPs) was found to be 3- to 5-fold higher with full-length Env than with the truncated forms, or than VLPs containing only Gag proteins, in primary monkey cells or various human cell lines. When cells expressing Env proteins in the absence of Gag were examined by immunoelectron microscopy, clusters of Env protein and membrane vesicles containing Env proteins were observed at cell surfaces. A low level of vesicles was released from cells expressing full-length Env, but about a 10-fold higher level was released in cells expressing a truncated form of Env [Env733(t)] in which the cytoplasmic domain is only 17 amino acids in length. Another truncated protein, Env718(t), with a short cytoplasmic tail of 3 aa, was also **incorporated** into VLPs at a 10-fold higher level than the

full-length Env protein and was more efficiently released in vesicles. The mature SU and TM proteins were predominantly **incorporated** into VLPs with full-length Env, but both cleaved and uncleaved precursor proteins were present in VLPs with truncated Env as well as in Env and Env(t) vesicles. A more prominent layer of spikes was seen by electron microscopy in VLPs with truncated Env than in VLPs containing full-length Env. These results indicate that truncated Env proteins have the ability to self-associate on the cell surface and are **assembled** into a more closely packed array than full-length Env, which could explain the preferential **incorporation** of Env proteins with short cytoplasmic tails into virions.

L51 ANSWER 14 OF 52 MEDLINE

ACCESSION NUMBER: 96211540 MEDLINE

DOCUMENT NUMBER: 96211540 PubMed ID: 8648742

TITLE: Chimeric hepatitis B virus core particles as probes for studying peptide-integrin interactions.

COMMENT: Erratum in: J Virol 1996 Aug;70(8):5740

AUTHOR: Chambers M A; Dougan G; Newman J; Brown F; Crowther J; Mould A P; Humphries M J; Francis M J; Clarke B; Brown A L; Rowlands D

CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, United Kingdom.

SOURCE: JOURNAL OF VIROLOGY, (1996 Jun) 70 (6) 4045-52.

Journal code: 0113724. ISSN: 0022-538X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199607

ENTRY DATE: Entered STN: 19960805

Last Updated on STN: 19980206

Entered Medline: 19960723

AB An RGD-containing epitope from the foot-and-mouth disease virus (FMDV) VP1 protein was inserted into the el loop of the hepatitis B virus core (HBc) protein. This chimeric protein was expressed at high levels in Escherichia coli and spontaneously assembled into **virus-like particles** which could be readily purified. These fusion particles elicited high levels of both enzyme-linked immunosorbent assay- and FMDV-neutralizing antibodies in guinea pigs. The chimeric particles bound specifically to cultured eukaryotic cells. Mutant particles carrying the tripeptide sequence RGE in place of RGD and the use of a competitive peptide, GRGDS, confirmed the critical involvement of the RGD sequence in this binding. The chimeric particles also bound to purified integrins, and inhibition by chain-specific anti-integrin monoclonal antibodies implicated alpha 5 beta 1 as a candidate cell **receptor** for both the **chimeric** particle and FMDV. Some serotypes of FMDV bound to beta 1 integrins in solid-phase assays, and the chimeric particles competed with FMDV for binding to susceptible eukaryotic cells. Thus, HBc particles may provide a simple, general system for exploring the interactions of specific peptide sequences with cellular receptors.

L51 ANSWER 15 OF 52 MEDLINE

ACCESSION NUMBER: 96204603 MEDLINE

DOCUMENT NUMBER: 96204603 PubMed ID: 8623547

TITLE: Proteolytic activity of human immunodeficiency virus Vpr- and Vpx-protease fusion proteins.

AUTHOR: Wu X; Liu H; Xiao H; Kappes J C

CORPORATE SOURCE: Department of Medicine, University of Alabama at Birmingham

35294, USA.
 CONTRACT NUMBER: AI31816 (NIAID)
 AI35282 (NIAID)
 P30-AI-27767 (NIAID)
 SOURCE: VIROLOGY, (1996 May 1) 219 (1) 307-13.
 Journal code: 0110674. ISSN: 0042-6822.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199606
 ENTRY DATE: Entered STN: 19960627
 Last Updated on STN: 19970203
 Entered Medline: 19960619

AB In addition to Gag, Pol, and Env, primate lentiviruses encode other virion-associated proteins, including Vpr, Vpx, and Vif. Vpr- and Vpx-staphylococcal nuclease and chloramphenicol acetyltransferase fusion proteins incorporate into human immunodeficiency virus (HIV) virions and retain **enzyme** activity when **expressed** in trans with HIV proviruses (Wu et al., J. Virol. 69, 3389, 1995). To explore whether the viral protease (PR) could be expressed as a proteolytically active fusion protein, the HIV PR coding region was fused in-frame with the HIV-2 vpx and HIV-1 vpr genes. Using a vaccinia virus-T7 expression system, the Vpx-PR fusion protein was expressed and formed homodimers. Coexpression with Pr55Gag demonstrated that Vpx-PR possessed Gag-specific proteolytic activity and inhibited the production of Gag **virus-like particles**. Trans-expression of a PR-Vpr fusion protein with HIV-1 provirus caused a profound reduction in viral protein expression and virion production. Importantly, the PR-Vpr fusion protein caused a similar level of inhibition and intracellular cleavage of Pr55Gag precursor protein when coexpressed with protease defective HIV-1 provirus. The inhibitory effect of PR-Vpr expression on virion production was markedly greater than that of PR alone. These results indicate that Vpr arguments the intracellular proteolytic activity of PR when expressed as a fusion protein and thus may be relevant for the expression of PR in intracellular immunization strategies against HIV infection. Moreover, the ability to express and package enzymatically active PR-Vpr fusion protein, independent of Gag/Pol, may provide a novel means to study enzyme function.

L51 ANSWER 16 OF 52 MEDLINE
 ACCESSION NUMBER: 96183925 MEDLINE
 DOCUMENT NUMBER: 96183925 PubMed ID: 8642705
 TITLE: Human immunodeficiency virus type 2 glycoprotein enhancement of particle budding: role of the cytoplasmic domain.
 AUTHOR: Ritter G D Jr; Yamshchikov G; Cohen S J; Mulligan M J
 CORPORATE SOURCE: Department of Medicine, University of Alabama at Birmingham, USA.
 CONTRACT NUMBER: AI-27767 (NIAID)
 AI-33784 (NIAID)
 SOURCE: JOURNAL OF VIROLOGY, (1996 Apr) 70 (4) 2669-73.
 Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199607
 ENTRY DATE: Entered STN: 19960726

Last Updated on STN: 19970203

Entered Medline: 19960718

AB Previous studies have shown that the glycoprotein cytoplasmic domains of human immunodeficiency virus type 2 (HIV-2) or simian immunodeficiency virus of macaques modulate biological activities of the viral glycoprotein complex, including syncytium formation, exterior glycoprotein conformation, and glycoprotein **incorporation** into budding virus particles. We have now utilized a recombinant expression system to study interactions of full-length or truncated HIV-2 glycoproteins with coexpressed HIV-2 Gag proteins which self-**assemble** and bud as **virus-like particles**. Interestingly, budding of HIV-2 **virus-like particles** from cells was enhanced 5- to 24-fold when Gag was coexpressed with the full-length HIV-2 glycoprotein, compared with Gag expressed either alone or with a truncated HIV-2 glycoprotein. The results obtained in this model system indicate that an additional effect of the lengthy cytoplasmic domain of the glycoprotein of HIV-2 is enhancement of particle budding. We speculate that the cytoplasmic domain of the viral glycoprotein of HIV-2 enhances budding by (i) potentiation of Gag structure or function or (ii) membrane modulation.

L51 ANSWER 17 OF 52 MEDLINE

ACCESSION NUMBER: 96095223 MEDLINE

DOCUMENT NUMBER: 96095223 PubMed ID: 8525638

TITLE: **Assembly of SIV virus-like particles** containing envelope proteins using a baculovirus expression system.

AUTHOR: Yamshchikov G V; Ritter G D; Vey M; Compans R W

CORPORATE SOURCE: Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, Georgia 30322, USA.

CONTRACT NUMBER: AI 28147 (NIAID)

AI 34242 (NIAID)

AI 35821 (NIAID)

SOURCE: VIROLOGY, (1995 Dec 1) 214 (1) 50-8.
Journal code: 0110674. ISSN: 0042-6822.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 199601

ENTRY DATE: Entered STN: 19960219

Last Updated on STN: 19960219

Entered Medline: 19960122

AB The requirements for SIV particle **assembly** and envelope **incorporation** were investigated using a baculovirus expression system. The Pr56gag precursor protein expressed under control of the polyhedrin promoter (pPolh) produced high levels of immature retrovirus-like particles (VLP) upon expression in Sf9 insect cells. To determine the optimal conditions for envelope protein (Env) **incorporation** into VLP, two recombinant baculoviruses expressing the SIV envelope protein under control of a very late pPolh or a hybrid late/very late capsid/polyhedrin (Pcap/polh) promoter and a recombinant expressing a truncated form of the SIV envelope protein (Envt) under the hybrid Pcap/polh promoter were compared. We have observed that utilization of the earlier hybrid promoter resulted in higher levels of Env expression on the cell surface and its **incorporation** into budding **virus** particles. We have also found that the Envt protein is transported to the cell surface of insect cells and **incorporated** into VLP more efficiently than full-length

Env. In addition, we examined the effect of coexpression of the protease furin, which has been implicated in the proteolytic cleavage of the Env precursor gp160 in mammalian cells. Coexpression of furin in insect cells resulted in more efficient proteolytic cleavage into gp120 and gp41, and the cleaved proteins were **incorporated** into VLP.

L51 ANSWER 18 OF 52 MEDLINE

ACCESSION NUMBER: 95395944 MEDLINE
 DOCUMENT NUMBER: 95395944 PubMed ID: 7666514
 TITLE: Characterization of deletion mutations in the capsid region of human immunodeficiency virus type 1 that affect particle formation and Gag-Pol precursor **incorporation**.
 AUTHOR: Srinivasakumar N; Hammarskjold M L; Rekosh D
 CORPORATE SOURCE: Myles H. Thaler Center for AIDS and Human Retrovirus Research, University of Virginia, Charlottesville 22908, USA.
 CONTRACT NUMBER: AI25721 (NIAID)
 AI30399 (NIAID)
 SOURCE: JOURNAL OF VIROLOGY, (1995 Oct) 69 (10) 6106-14.
 Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199510
 ENTRY DATE: Entered STN: 19951020
 Last Updated on STN: 19970203
 Entered Medline: 19951012

AB The core of human immunodeficiency virus type 1 is derived from two precursor polyproteins, Pr55gag and Pr160gag-pol. The Gag precursor can **assemble** into immature **virus-like particles** when expressed by itself, while the Gag-Pol precursor lacks particle-forming ability. We have shown previously that the Gag precursor is able to "rescue" the Gag-Pol precursor into **virus-like particles** when the two polyproteins are expressed in the same cell by using separate simian virus 40-based plasmid expression vectors. To understand this interaction in greater detail, we have made deletion mutations in the capsid-coding regions of Gag- and Gag-Pol-expressing plasmids and assayed for the abilities of these precursors to **assemble** into **virus-like particles**. When we tested the abilities of Gag-Pol precursors to be **incorporated** into particles of Gag by coexpressing the precursors, we found that mutant Gag-Pol precursors lacking a conserved region in retroviral capsid proteins, the major homology region (MHR), were excluded from wild-type Gag particles. Mutant precursors lacking MHR were also less efficient in processing the Gag precursor in trans. These results suggest that the MHR is critical for interactions between Gag and Gag-Pol molecules. In contrast to these results, expression of mutated Gag precursors alone showed that deletions in the capsid region, including those which removed the MHR, reduced the efficiency of particle formation by only 40 to 50%. The mutant particles, however, were clearly lighter than the wild type in sucrose density gradients. These results indicate that the requirements for Gag particle formation differ from the ones essential for efficient **incorporation** of the Gag-Pol precursor into these particles.

L51 ANSWER 19 OF 52 MEDLINE

ACCESSION NUMBER: 95287455 MEDLINE
 DOCUMENT NUMBER: 95287455 PubMed ID: 7769663

TITLE: **Incorporation** of pseudorabies virus gD into human immunodeficiency virus type 1 Gag particles produced in baculovirus-infected cells.
 AUTHOR: Garnier L; Ravallec M; Blanchard P; Chaabihi H; Bossy J P; Devauchelle G; Jestin A; Cerutti M
 CORPORATE SOURCE: Laboratoire de Pathologie Comparee, Centre National de la Recherche Scientifique UA 1184, France.
 SOURCE: JOURNAL OF VIROLOGY, (1995 Jul) 69 (7) 4060-8.
 Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199507
 ENTRY DATE: Entered STN: 19950713
 Last Updated on STN: 19970203
 Entered Medline: 19950706

AB The human immunodeficiency virus type 1 (HIV-1) Pr55gag precursors were previously shown to **assemble** and bud efficiently as noninfectious **virus-like particles** (VLPs) when expressed in baculovirus-infected insect cells. In this study, we examined the abilities of foreign antigens to be **incorporated** on the outer surface of HIV-1 Gag particles. We have used a dual recombinant baculovirus, expressing the HIV-1 **Gag gene** and gD **gene** under the control of the P10 and polyhedrin promoters, respectively, to obtain hybrid VLPs. Transmission electron microscopy of insect cells infected with the dual recombinant revealed very large aggregates of particles budding from the cell membrane. The release of VLPs into the culture medium was clearly different for a recombinant baculovirus producing solely HIV-1 Gag, for which particles were uniformly distributed all around the cell surface. Biochemical analysis of hybrid particles indicated that glycoprotein gD was packaged into HIV-1 Gag VLPs. Moreover, the carboxy-terminal p6 region of Gag polyprotein and the glycoprotein gD intracytoplasmic domain were not required for gD **incorporation**. The experiments described here clearly demonstrate that glycoprotein gD can be packaged with HIV-1 Gag particles and released from insect cells.

L51 ANSWER 20 OF 52 MEDLINE
 ACCESSION NUMBER: 94365918 MEDLINE
 DOCUMENT NUMBER: 94365918 PubMed ID: 8083957
 TITLE: Localization of the Vpx packaging signal within the C terminus of the human immunodeficiency virus type 2 Gag precursor protein.
 AUTHOR: Wu X; Conway J A; Kim J; Kappes J C
 CORPORATE SOURCE: Department of Medicine, University of Alabama at Birmingham 35294.
 CONTRACT NUMBER: AI31816 (NIAID)
 P30-AI-27767-06 (NIAID)
 SOURCE: JOURNAL OF VIROLOGY, (1994 Oct) 68 (10) 6161-9.
 Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199410
 ENTRY DATE: Entered STN: 19941021
 Last Updated on STN: 19970203
 Entered Medline: 19941013

AB Viral protein X (Vpx) is a human immunodeficiency virus type 2 (HIV-2) and simian immunodeficiency virus accessory protein that is packaged into virions in molar amounts equivalent to Gag proteins. To delineate the processes of virus **assembly** that mediate Vpx packaging, we used a recombinant vaccinia virus-T7 RNA polymerase system to facilitate Gag protein expression, particle **assembly**, and extracellular release. HIV genes were placed under control of the bacteriophage T7 promoter and transfected into HeLa cells expressing T7 RNA polymerase. Western immunoblot analysis detected p55gag and its cleavage products p39 and p27 in purified particles derived by expression of gag and gag-pol, respectively. In trans expression of vpx with either HIV-2 gag or gag-pol gave rise to **virus-like particles** that contained Vpx in amounts similar to that detected in HIV-2 virus produced from productively infected T cells. Using C-terminal deletion and truncation mutants of HIV-2 Gag, we mapped the p15 coding sequence for determinants of Vpx packaging. This analysis revealed a region (residues 439 to 497) downstream of the nucleocapsid protein (NC) required for **incorporation** of Vpx into virions. HIV-1/HIV-2 gag chimeras were constructed to further characterize the requirements for **incorporation** of Vpx into virions. Chimeric HIV-1/HIV-2 Gag particles consisting of HIV-1 p17 and p24 fused in frame at the C terminus with HIV-2 p15 effectively **incorporate** Vpx, while chimeric HIV-2/HIV-1 Gag particles consisting of HIV-2 p17 and p27 fused in frame at the C terminus with HIV-1 p15 do not. Expression of a 68-amino-acid sequence of HIV-2 containing residues 439 to 497 fused to the coding regions of HIV-1 p17 and p24 also produced **virus-like particles** capable of packaging Vpx in amounts similar to that of full-length HIV-2 Gag. Sucrose gradient analysis confirmed particle association of Vpx and Gag proteins. These results demonstrate that the HIV-2 Gag precursor (p55) regulates **incorporation** of Vpx into virions and indicates that the packaging signal is located within residues 439 to 497.

L51 ANSWER 21 OF 52 MEDLINE
 ACCESSION NUMBER: 94335114 MEDLINE
 DOCUMENT NUMBER: 94335114 PubMed ID: 8057473
 TITLE: Efficiency and selectivity of RNA packaging by Rous sarcoma virus Gag deletion mutants.
 AUTHOR: Sakalian M; Wills J W; Vogt V M
 CORPORATE SOURCE: Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14850.
 CONTRACT NUMBER: CA-20081 (NCI)
 CA-47482 (NCI)
 SOURCE: JOURNAL OF VIROLOGY, (1994 Sep) 68 (9) 5969-81.
 Journal code: 0113724. ISSN: 0022-538X.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199409
 ENTRY DATE: Entered STN: 19940920
 Last Updated on STN: 19970203
 Entered Medline: 19940909

AB In all retrovirus systems studied, the leader region of the RNA contains a cis-acting sequence called psi that is required for packaging the viral RNA genome. Since the pol and env genes are dispensable for formation of RNA-containing particles, the **gag gene** product must have an RNA binding domain(s) capable of recognizing psi. To gain information about which portion(s) of Gag is required for RNA packaging in

the avian sarcoma and leukemia virus system, we utilized a series of gag deletion mutants that retain the ability to **assemble virus-like particles**. COS cells were cotransfected with these mutant DNAs plus a tester DNA containing psi, and **incorporation** of RNA into particles were measured by RNase protection. The efficiency of packaging was determined by normalization of the amount of psi+ RNA to the amount of Gag protein released in **virus-like particles**. Specificity of packaging was determined by comparisons of psi+ and psi- RNA in particles and in cells. The results indicate that much of the MA domain, much of the p10 domain, half of the CA domain, and the entire PR domain of Gag are unnecessary for efficient packaging. In addition, none of these deleted regions is needed for specific selection of the psi RNA. Deletions within the NC domain, as expected, reduce or eliminate both the efficiency and the specificity of packaging. Among mutants that retain the ability to package, a deletion within the CA domain (which includes the major homology region) is the least efficient. We also examined particles of the well-known packaging mutant SE21Q1b. The data suggest that the random RNA packaging behavior of this mutant is not due to a specific defect but rather is the result of the cumulative effect of many point mutations throughout the **gag gene**.

L51 ANSWER 22 OF 52 MEDLINE

ACCESSION NUMBER: 94244606 MEDLINE
 DOCUMENT NUMBER: 94244606 PubMed ID: 7514530
 TITLE: Reverse transcriptase activity of an intron encoded polypeptide.
 AUTHOR: Fassbender S; Bruhl K H; Ciriacy M; Kuck U
 CORPORATE SOURCE: Lehrstuhl fur Allgemeine Botanik, Ruhr-Universitat Bochum, Germany.
 SOURCE: EMBO JOURNAL, (1994 May 1) 13 (9) 2075-83.
 Journal code: 8208664. ISSN: 0261-4189.
 PUB. COUNTRY: ENGLAND: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199406
 ENTRY DATE: Entered STN: 19940629
 Last Updated on STN: 19990129
 Entered Medline: 19940617

AB A number of group II introns from eukaryotic organelles and prokaryotes contain open reading frames for polypeptides with homology to retroviral reverse transcriptases (RTs). We have used the yeast transposon (Ty) system to express ORFs for RTs from eukaryotic organelles. This includes the mitochondrial coxI intron 11 from the fungus *Podospora anserina*, the plastid petD intron from the alga *Scenedesmus obliquus* and the mitochondrial RTL gene from the alga *Chlamydomonas reinhardtii*. The ORFs were fused with the TYA ORF from the yeast retrotransposon Ty to produce **virus-like particles** in the recipient strains with detectable amounts of the RT-like **polypeptides**. Analysis of the **heterologous** gene products revealed biochemical evidence that the *P. anserina* intron encodes an RNA-directed DNA polymerase with properties typically found for RTs of viral or retrotransposable origin. In vitro assays showed that the intron encoded RT is sensitive to RT inhibitors such as N-ethylmaleimide and dideoxythymidine triphosphate but is insensitive against the DNA polymerase inhibitor aphidicolin. The direct biochemical evidence provided here supports the idea that intron encoded RTs are involved in intron transposition events.

L51 ANSWER 23 OF 52 MEDLINE

ACCESSION NUMBER: 94173942 MEDLINE
 DOCUMENT NUMBER: 94173942 PubMed ID: 8127909
 TITLE: RGD sequence of foot-and-mouth disease virus is essential for infecting cells via the natural receptor but can be bypassed by an antibody-dependent enhancement pathway.
 AUTHOR: Mason P W; Rieder E; Baxt B
 CORPORATE SOURCE: Plum Island Animal Disease Center, U.S. Department of Agriculture, Greenport, NY 11944.
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Mar 1) 91 (5) 1932-6. Journal code: 7505876. ISSN: 0027-8424.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199404
 ENTRY DATE: Entered STN: 19940420
 Last Updated on STN: 19970203
 Entered Medline: 19940411

AB Foot-and-mouth disease virus appears to initiate infection by binding to cells at an Arg-Gly-Asp (RGD) sequence found in the flexible beta G-beta H loop of the viral capsid protein VP1. The role of the RGD sequence in attachment of virus to cells was tested by using synthetic full-length viral RNAs mutated within or near the RGD sequence. Baby hamster kidney (BHK) cells transfected with three different RNAs carrying mutations bordering the RGD sequence produced infectious viruses with wild-type plaque morphology; however, one of these mutant viruses bound to cells less efficiently than wild type. BHK cells transfected with RNAs containing changes within the RGD sequence produced noninfectious particles indistinguishable from wild-type virus in terms of sedimentation coefficient, binding to monoclonal antibodies, and protein composition. These **virus-like particles** are defined as ads- viruses, since they were unable to adsorb to and infect BHK cells. These mutants were defective only in cell binding, since antibody-complexed ads- viruses were able to infect Chinese hamster ovary cells **expressing** an immunoglobulin Fc **receptor**. These results confirm the essential role of the RGD sequence in binding of foot-and-mouth disease virus to susceptible cells and demonstrate that the natural cellular receptor for the virus serves only to bind virus to the cell.

L51 ANSWER 24 OF 52 MEDLINE

ACCESSION NUMBER: 93276541 MEDLINE
 DOCUMENT NUMBER: 93276541 PubMed ID: 8503172
 TITLE: **Assembly** of the matrix protein of simian immunodeficiency **virus** into **virus-like particles**.
 AUTHOR: Gonzalez S A; Affranchino J L; Gelderblom H R; Burny A
 CORPORATE SOURCE: Department of Molecular Biology, University of Brussels (ULB), Belgium.
 SOURCE: VIROLOGY, (1993 Jun) 194 (2) 548-56. Journal code: 0110674. ISSN: 0042-6822.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199306
 ENTRY DATE: Entered STN: 19930716

Last Updated on STN: 19970203
Entered Medline: 19930625

AB To obtain a better understanding of the processes of **assembly** and morphogenesis of simian immunodeficiency virus (SIV), recombinant vaccinia viruses containing regions of the gag-pol open reading frame were constructed and their intracellular expression as well as the ability of the Gag polypeptides to be released into the culture medium as constituents of **virus-like particles** were studied. Biochemical and electron microscopy analyses of cells infected with a recombinant expressing only the SIV matrix (MA) domain of the Gag polyprotein (v-pl7 gag) showed that this protein self-**assembles** into 100-nm **virus-like particles** which are released into the culture medium. Interestingly, coexpression of SIV MA and Env proteins resulted in **incorporation** of gp120 and gp41 proteins into the recombinant pl7-made particles. In addition when a positively charged domain of SIV MA (residues 26-33), which is highly conserved among all HIV and SIV MA proteins, was mutated into an acidic region, particle release was abolished without affecting protein expression, processing, or stability. Further characterization of the phenotype of this mutant by electron microscopy indicated that this mutant was blocked at the stage of **assembly**. These results suggest that SIV MA protein, along with its function in myristic acid-mediated membrane targeting, has intrinsic information for self-**assembly** as well as **incorporation** of viral Env glycoproteins into particles.

L51 ANSWER 25 OF 52 MEDLINE

ACCESSION NUMBER: 93188179 MEDLINE

DOCUMENT NUMBER: 93188179 PubMed ID: 8445731

TITLE: Requirements for **incorporation** of Pr160gag-pol from human immunodeficiency virus type 1 into **virus-like particles**.

AUTHOR: Smith A J; Srinivasakumar N; Hammarskjold M L; Rekosh D

CORPORATE SOURCE: Department of Microbiology, State University of New York, Buffalo 14214.

CONTRACT NUMBER: AI-25721 (NIAID)
AI-30399 (NIAID)

SOURCE: JOURNAL OF VIROLOGY, (1993 Apr) 67 (4) 2266-75.
Journal code: 0113724. ISSN: 0022-538X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 199304

ENTRY DATE: Entered STN: 19930416

Last Updated on STN: 19970203

Entered Medline: 19930408

AB The roles of the human immunodeficiency virus precursor polyproteins Pr55gag and Pr160gag-pol in viral core **assembly** were studied in CMT3-COS cells. To do this, the precursors were expressed separately by using a simian virus 40 late replacement vector system described previously. Consistent with previously published data, our results show that the Pr55gag precursor, when expressed alone, was able to form particles which had an immature morphology and that particle formation required the presence of a myristate addition signal at the amino terminus of the precursor. In contrast, the Pr160gag-pol precursor was not able to form particles when expressed alone, although it still underwent proteolytic processing. Coexpression of the two precursor polyproteins from separate vectors in the same cell resulted in processing of the Pr55gag in trans by the protease embedded in Pr160gag-pol and the

formation of **virus-like particles** containing the products of both precursors. Proteolytic processing occurred independently of the presence of a functional myristate addition signal on either precursor. On the other hand, removal of myristate from one or the other precursor had nonreciprocal effects on virus particle formation. Cells expressing Pr55gag lacking myristate and Pr160gag-pol containing it did not produce particles. Cells expressing a myristylated Pr55gag and unmyristylated Pr160gag-pol still produced **virus-like particles** which contained nearly normal amounts of Pr160gag-pol. The results suggest that the **incorporation** of Pr160gag-pol into particles is largely determined by intermolecular protein-protein interactions between the two precursor polypeptides.

L51 ANSWER 26 OF 52 MEDLINE

ACCESSION NUMBER: 93139775 MEDLINE

DOCUMENT NUMBER: 93139775 PubMed ID: 8093711

TITLE: Host cell membrane proteins on human immunodeficiency virus type 1 after in vitro infection of H9 cells and blood mononuclear cells. An immuno-electron microscopic study.

AUTHOR: Meerloo T; Sheikh M A; Bloem A C; de Ronde A; Schutten M; van Els C A; Roholl P J; Joling P; Goudsmit J; Schuurman H J

CORPORATE SOURCE: Department of Pathology, University Hospital, Utrecht, The Netherlands.

SOURCE: JOURNAL OF GENERAL VIROLOGY, (1993 Jan) 74 (Pt 1) 129-35. Journal code: 0077340. ISSN: 0022-1317.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 199302

ENTRY DATE: Entered STN: 19930312

Last Updated on STN: 19970203

Entered Medline: 19930222

AB Human immunodeficiency virus type 1 (HIV-1)-infected H9 and blood mononuclear cells (MNCs) were studied by immunogold electron microscopy for the presence of HIV-1 gag p24 protein, env gp41 and gp120 proteins, and host cell molecules CD4, CD11a, CD25, CD54, CD63, HLA class I and HLA-DR. Uninfected H9 cells and MNC membranes labelled for CD4, HLA class I and class II, and, at low density, CD11a and CD54; lysosomal structures in the cytoplasm labelled for CD63. The infected cell surface showed immunolabelling for HIV-1 proteins, as did budding particle-like structures. Immunogold labelling of the cell membrane for CD4 was almost non-existent. The level of immunolabelling for CD11a and CD54 on infected cells was greater than that on uninfected cells; this is presumably related to a state of activation during **virus** synthesis. Budding **particle-like** structures and free virions in the intercellular space were immunogold-labelled for all host cell markers investigated. This was confirmed by double immunogold labelling using combinations of HIV-1 gag p24 labelling and labelling for the respective host cell molecule. We conclude that virions generated in HIV-1-infected cells concentrate host-derived molecules on their envelope. Also molecules with a prime function in cellular adhesion concentrate on the virion.

L51 ANSWER 27 OF 52 MEDLINE

ACCESSION NUMBER: 93134805 MEDLINE

DOCUMENT NUMBER: 93134805 PubMed ID: 8421902

TITLE: Analysis of protein expression and **virus-like particle** formation in mammalian cell

lines stably expressing HIV-1 **gag** and **env** **gene** products with or without active HIV proteinase.

AUTHOR: Krausslich H G; Ochsenbauer C; Traenckner A M; Mergener K; Facke M; Gelderblom H R; Bosch V

CORPORATE SOURCE: Angewandte Tumorstudiologie, Deutsches Krebsforschungszentrum, Heidelberg.

SOURCE: VIROLOGY, (1993 Feb) 192 (2) 605-17.
Journal code: 0110674. ISSN: 0042-6822.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; AIDS

ENTRY MONTH: 199302

ENTRY DATE: Entered STN: 19930226
Last Updated on STN: 19970203
Entered Medline: 19930217

AB Cell lines stably releasing noninfectious **virus-like particles** containing wild type or mutant gene products represent useful tools for a biochemical, immunological, and structural analysis of virus **assembly**. Human immunodeficiency virus (HIV) type 1 **gag** and **env** **gene** products were transiently and stably expressed in mammalian cells and the formation of **virus-like particles incorporating** viral glycoproteins was analyzed. Transient cotransfection of plasmids directing the synthesis of **gag** and **env** **gene** products yielded efficient release of particles but specific **incorporation** of HIV glycoproteins was not detected. A stable cell line expressing wild type HIV-1 glycoproteins was generated and transient transfection of this cell line with **gag**-encoding constructs led to the release of **virus-like particles incorporating** HIV surface and transmembrane glycoproteins. Attempts to establish stable cell lines expressing wild type HIV **gag** and **pol** **genes** were unsuccessful and only highly unstable lines primarily expressing uncleaved precursor polyproteins were obtained. This result appears to be caused by the cytotoxic effects of the viral proteinase since stable lines were readily selected after transfection of constructs either encoding an inactive mutant of the proteinase or a mutated frameshift signal which prevented expression of the **pol** reading frame. Stable coexpression of uncleaved **Gag** polyprotein and wild type **env** gene products yielded efficient release of immature **virus-like particles incorporating** HIV glycoproteins. Electron micrographs revealed lentiviral budding structures with the typical surface projections of viral glycoprotein **oligomers**.

L51 ANSWER 28 OF 52 MEDLINE

ACCESSION NUMBER: 90151906 MEDLINE

DOCUMENT NUMBER: 90151906 PubMed ID: 1689252

TITLE: The biogenesis and function of eukaryotic porins.

AUTHOR: Dihanich M

CORPORATE SOURCE: Friedrich-Miescher-Institute, Basel, Switzerland.

SOURCE: EXPERIENTIA, (1990 Feb 15) 46 (2) 146-53. Ref: 77
Journal code: 0376547. ISSN: 0014-4754.

PUB. COUNTRY: Switzerland

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199003
 ENTRY DATE: Entered STN: 19900601
 Last Updated on STN: 19960129
 Entered Medline: 19900329

AB Like most other mitochondrial proteins porin is synthesized in the cytosol and imported posttranslationally into the outer mitochondrial membrane. This transport follows the general rules for mitochondrial protein import with a few aberrations: a) porin contains an uncleaved NH2-terminal signal sequence, b) also its carboxyterminus might be involved in the import process, and c) this transport does not seem to require a membrane potential $\Delta\psi$, although it is ATP-dependent. Most likely the actual import step occurs at contact sites between the outer and the inner mitochondrial membrane and involves at least one receptor protein. Although porin is known to be the major gate through the outer mitochondrial membrane, its absence only causes transient respiratory problems in yeast cells. This could mean a) that there is a bypass for some mitochondrial functions in the cytosol and/or b) that there are alternative channel proteins in the outer membrane. The first idea is supported by the overexpression of cytosolic **virus-like particles** in yeast cells lacking porin and the second by the occurrence of residual pore activity in mitochondrial outer membrane purified from porinless mutant cells.

L51 ANSWER 29 OF 52 MEDLINE
 ACCESSION NUMBER: 90148545 MEDLINE
 DOCUMENT NUMBER: 90148545 PubMed ID: 2559750
 TITLE: A novel method for the purification of HIV-1 p24 protein from hybrid Ty **virus-like particles** (Ty-VLPs).
 AUTHOR: Gilmour J E; Senior J M; Burns N R; Esnouf M P; Gull K; Kingsman S M; Kingsman A J; Adams S E
 CORPORATE SOURCE: British Bio-technology Ltd., Cowley, UK.
 SOURCE: AIDS, (1989 Nov) 3 (11) 717-23.
 Journal code: 8710219. ISSN: 0269-9370.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals; AIDS
 ENTRY MONTH: 199003
 ENTRY DATE: Entered STN: 19900601
 Last Updated on STN: 19990129
 Entered Medline: 19900316

AB The self-**assembly** properties of a protein encoded by the yeast retrotransposon Ty can be exploited to produce large amounts of recombinant, particulate fusion proteins as hybrid Ty **virus-like particles** (Ty-VLPs). This system has now been adapted to allow the release of the additional protein by **incorporation** of a protease cleavage site between the yeast carrier protein and the protein of interest. The purification of the additional protein is facilitated by exploiting the ease with which Ty-VLPs can be purified from other yeast cell components due to their particulate nature. We have used this modified system to produce hybrid particles containing the HIV-1 p24 protein downstream of the recognition sequence for the blood coagulation factor Xa. The p24 was released from the particles by proteolytic cleavage and rapidly separated from the residual particulate material using centrifugation and standard chromatography techniques. This procedure has been used to purify milligram quantities of HIV-1 p24 protein that reacts with anti-p24 sera and elicits the production of p24-specific antibodies in experimental

animals.

L51 ANSWER 30 OF 52 MEDLINE
ACCESSION NUMBER: 77191070 MEDLINE
DOCUMENT NUMBER: 77191070 PubMed ID: 325296
TITLE: Hepatitis B core and surface antigens in liver tissue.
Light and electron microscopic localization by the
peroxidase-labeled antibody method.
AUTHOR: Yamada G; Nakane P K
SOURCE: LABORATORY INVESTIGATION, (1977 Jun) 36 (6) 649-59.
Journal code: 0376617. ISSN: 0023-6837.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197707
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19900314
Entered Medline: 19770723

AB Hepatitis B core antigen (HBcAg) and hepatitis B surface antigen (HBsAg) were localized in human liver tissues by the peroxidase-labeled antibody method at the light and electron microscopic levels. Several methods of fixation, staining, and inhibition of endogenous peroxidase activity were studied. The periodate-lysine-paraformaldehyde fixative effectively preserved the tissue structure and the antigenicity of both antigens, and the peroxidase-labeled Fab' fraction of IgG penetrated well into hepatocytes. HBcAg was present in nuclei, or cytoplasm of hepatic cells, or both. In nuclei, the antigen was found both in **virus-like particles** of approximately 20 nm. diameter and in nuclear ground substance. In the cytoplasm, the antigen was found on membrane-bound ribosomes and free polysomes, and also in the ground substance of the cytosol near ribosomes and around nuclear **membranes**, especially near nuclear **pores**. HBcAg-positive **virus-like particles** were also demonstrated sparsely or in clusters in the cytoplasm. HBsAg was not present in nuclei but was found in the perinuclear space and in cisternae of endoplasmic reticulum, and on nuclear, endoplasmic reticulum, and cell membranes of hepatic cells. HBsAg-positive 25- to 30-nm. wide tubular forms, round particles (probably cross-sections of tubular forms), and a few large particles of 40 to 50 nm. diameter were seen in cisternae. Such HBsAg-positive particles were also present in the intercellular space and in Disse's space. These findings suggest that HBcAg produced on the cytoplasmic ribosomes migrates through nuclear pores to the nucleus and is assembled into core particles there. These particles may then move through nuclear pores to the cytoplasm where they are invested with HBsAg-positive membrane in cisternae of endoplasmic reticulum or as they enter the endoplasmic reticulum. These virus particles are then released together with other HBsAg-positive forms into the intercellular space by reversed phagocytosis.

L51 ANSWER 31 OF 52 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:332632 HCAPLUS
DOCUMENT NUMBER: 136:336197
TITLE: **Virus like particles,**
their preparation and their use in drug screening and
functional genomics
INVENTOR(S): **Hunt, Nicholas**
PATENT ASSIGNEE(S): Germany
SOURCE: U.S. Pat. Appl. Publ., 60 pp., Cont.-in-part of U.S.

Ser. No. 673,257.

CODEN: USXXCO

DOCUMENT TYPE:

Patent

LANGUAGE:

English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002052040	A1	20020502	US 2000-750185	20001229
WO 2001002551	A2	20010111	WO 2000-EP6144	20000626
WO 2001002551	A3	20011108		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.:

EP 1999-112451	A	19990630
EP 2000-106109	A	20000321
US 2000-191318P	P	20000321
EP 2000-110363	A	20000515
WO 2000-EP6144	W	20000626
US 2001-673257	A2	20011002
US 1999-141268P	P	19990630

AB The invention relates to **virus like particles**, their prepn. and their use preferably in pharmaceutical screening and functional genomics. The invention further provides a variety of assay formats to be used with said **virus like particles**. In a first aspect the invention provides a method to selectively incorporate or encapsulate proteinaceous target mols. into **virus like particles** (VLP5). Target mols. are co-expressed in recombinant cells together with signal mols. It is possible to generate a homogeneous population of VLPs in which a functional target protein of choice is expressed either within the lipid bilayer of an enveloped **VLP** or within the capsid of a naked or enveloped **VLP**. It is also possible to encapsulate target proteins within the **VLP**. These reactions are mediated by the specific interaction with a signaling protein. The incorporation/encapsulation of the resp. target proteins is preferably achieved by utilization of a signal mol. with a specific concatemeric protein sequence which interacts specifically and with high affinity with a complementary concatemeric tag located at either the carboxy or amino terminal end of the resp. target protein. When both of these modified proteins (signal and target) are expressed within the same host cell, then the expressed protein products assoc. with one another via the specific tags. This interaction results in a preferred embodiment in the translocation of the resp. complexes to the cell membrane in high concns. where they are extruded from the cells via a budding process similar to the release of mature **virus** particles. With respect to the second amino acid sequence of the signal mol., it is preferred that it comprises at least a fragment of a **virus** capsid or envelope protein, or a precursor of a **virus** capsid or envelope protein, or a mutant of a **virus** capsid or envelope protein. It is also possible to utilize a second amino acid sequence of said signal mol. which is encoded by at least a fragment of a retrotransposon, in

particular a Ty element in yeast, a copia element in insects, a copia-like element in insects, VL 30 in mice, or an IAP gene in mice. The invention is exemplified by displaying **G-protein coupled receptors**, or human epidermal growth factor receptor (EGFR), or endothelin receptors to allow identification of gene products interfering with protein-protein interactions within the cell.

L51 ANSWER 32 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:31628 HCAPLUS

DOCUMENT NUMBER: 134:96212

TITLE: **Virus like particles,**
their preparation and their use preferably in
pharmaceutical screening and functional genomics

INVENTOR(S): **Hunt, Nicholas**

PATENT ASSIGNEE(S): Evotec Biosystems A.-G., Germany

SOURCE: PCT Int. Appl., 125 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001002551	A2	20010111	WO 2000-EP6144	20000626
WO 2001002551	A3	20011108		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
EP 1187928	A2	20020320	EP 2000-949236	20000630
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
US 2002052040	A1	20020502	US 2000-750185	20001229
EP 1219705	A1	20020703	EP 2000-128686	20001229
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR			
PRIORITY APPLN. INFO.:			EP 1999-112451	A 19990630
			US 1999-141268P	P 19990630
			EP 2000-106109	A 20000321
			EP 2000-110363	A 20000515
			US 2000-191318P	P 20000321
			WO 2000-EP6144	W 20000626
			US 2001-673257	A2 20011002

AB The invention relates to **virus like particles (VLP)**, their prepn. and their use in pharmaceutical screening and functional genomics. The **VLP** can display the target protein within the its capsid through either strong specific interaction of a mol. peptide tag covalently attached to the C-terminus of the signal protein (Gag) with a complementary specific peptide tag assocd. with the target of interest or by direct covalent fusion of the Gag protein with the target protein/peptide of interest. The Gag-tag fusion protein is co-expressed in a cellular system with the resp. mol. of interest which also carries a specific peptide tag either within the mol. or at either the N- or

C-terminus. Expression of the modified Gag protein in the resp. host cells results in the accumulation of the Gag protein at the plasma membrane due to signals present within the N-terminal portion of the Gag protein. High concns. of this protein at the plasma membrane results in a budding process in which VLPs are released into the extracellular milieu. If the target protein carrying the complementary tag is expressed in the same cell and is concd. in the intracellular compartments then the specific interaction with the tagged Gag protein results in the cotransport of the target to the plasma membrane and subsequent incorporation into the released VLPs. The invention further provides a variety of assay formats to be used with said **virus like particles**. The invention is exemplified by displaying **G-protein coupled receptors**, or human epidermal growth factor receptor (EGFR), or endothelin receptors to allow identification of gene products interfering with protein-protein interactions within the cell.

L51 ANSWER 33 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:5331 HCAPLUS

DOCUMENT NUMBER: 132:136156

TITLE: Self-assembly of the infectious bursal disease virus capsid

protein, rVP2, expressed in insect cells and purification of immunogenic chimeric rVP2H particles by immobilized metal-ion affinity chromatography

AUTHOR(S): Wang, Min-Ying; Kuo, Yung-Yan; Lee, Meng-Shiou; Doong, Shyue-Ru; Ho, Ji-Yi; Lee, Long-Huw

CORPORATE SOURCE: Graduate Institute of Agricultural Biotechnology
National Chung Hsing University, Taichung, 40227,
Taiwan

SOURCE: Biotechnology and Bioengineering (2000), 67(1),
104-111

CODEN: BIBIAU; ISSN: 0006-3592

PUBLISHER: John Wiley & Sons, Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A gene encoding a structural protein (VP2) of a local strain (P3009) of infectious bursal disease virus (IBDV) was cloned and expressed using the baculovirus expression system to develop a subunit vaccine against IBDV infection in Taiwan. The expressed rVP2 proteins formed particles of approx. 20-30 nm in diam. Those particles were partially purified employing sucrose d. gradient ultracentrifugation, and the purified particles were recognized by a monoclonal antibody against the VP2 protein of IBDV P3009. To facilitate the purifn. of the particles, the VP2 protein was engineered to **incorporate** a metal ion binding site (His)6 at its C-terminus. The chimeric rVP2H proteins also formed particles, which could be affinity-purified in one step with immobilized metal ions (Ni²⁺). Particle formation was confirmed by direct observation under the electron microscope. The prodn. level of rVP2H protein was detd. to be 20 mg/L in a batch culture of Hi-5 cells by quantifying the concn. of the purified proteins. The chicken protection assay was performed to evaluate the immunogenicity of the rVP2H protein. When susceptible chickens were inoculated with the recombinant rVP2H proteins (40 .mu.g/bird), virus-neutralizing antibodies were induced, thereby conferring a high level of protection against the challenge of a very virulent strain of IBDV. In conclusion, the most significant finding in this work is that both of the expressed rVP2 and rVP2H proteins can form a particulate structure capable of inducing a strong immunol. response in a vaccinated chicken.

REFERENCE COUNT: 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L51 ANSWER 34 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:723798 HCAPLUS

DOCUMENT NUMBER: 129:340530

TITLE: Corona **virus-like particles** with altered cellular specificity for infection

INVENTOR(S): Rottier, Petrus Josephus Marie

PATENT ASSIGNEE(S): Universiteit Utrecht, Neth.

SOURCE: PCT Int. Appl., 38 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9849195	A1	19981105	WO 1998-NL237	19980429
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9873507	A1	19981124	AU 1998-73507	19980429
PRIORITY APPLN. INFO.:			EP 1997-201292	19970429
			WO 1998-NL237	19980429

AB The present invention provides **virus-like particles** (VLPs) derived from corona viruses which are modified for their envelope and/or nucleocapsid protein expression. In one example, the ectodomain of the coronavirus spike protein was replaced with that corresponding to mouse hepatitis virus and a change in cellular tropism was obsd. The VLPs can be used as systems for the targeted delivery of therapeutic agents (e.g. CD4, antibodies, drugs) or can be used as vaccine or as antigen in diagnostic tests.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L51 ANSWER 35 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:325338 HCAPLUS

DOCUMENT NUMBER: 127:48627

TITLE: **Virus-like particle**
-induced fusion from without in tissue culture cells:
role of outer-layer proteins VP4 and VP7

AUTHOR(S): Gilbert, Joanna M.; Greenberg, Harry B.

CORPORATE SOURCE: Dep. Microbiol. and Immunology and Dep. Medicine,
Division Gastroenterology, Stanford Univ. School
Medicine, Stanford, CA, 94305, USA

SOURCE: Journal of Virology (1997), 71(6), 4555-4563

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors recently described an assay that measures fusion from without

induced in tissue culture cells by rotavirus, a nonenveloped, triple-protein-layered member of the Reoviridae family. The conditions required for syncytium formation are similar to those for viral penetration of the plasma membrane during the course of viral infection of host cells, as the presence of the outer-layer proteins VP4 and VP7 and the cleavage of VP4 are required. Here the authors present evidence that **virus-like particles** (VLPs) produced in *Spodoptera frugiperda* Sf-9 cells from recombinant baculoviruses expressing the four structural proteins of rotavirus can induce cell-cell fusion to the same extent as native rotavirus. This **VLP**-mediated fusion activity was dependent on trypsinization of VP4, and the strain-specific phenotype of individual VP4 mols. was retained in the syncytium assay similar to what has been seen with reassortant rotaviruses. The authors show that intact rotavirus and VLPs induce syncytia with cells that are permissive to rotavirus infection whereas nonpermissive cells are refractory to syncytium formation. This finding further supports the hypothesis that the syncytium assay accurately reflects very early events involved in viral infection and specifically the events related to viral entry into the cell. The results also demonstrate that neither viral replication nor rotavirus proteins other than VP2, VP6, VP4, and VP7 are required for fusion and that both VP4 and VP7 are essential. The combination of a cell-cell fusion assay and the availability of recombinant VLPs will permit the authors' to dissect the mechanisms of rotavirus penetration into host cells.

L51 ANSWER 36 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:548780 HCAPLUS

DOCUMENT NUMBER: 125:219349

TITLE: Immunologic and ultrastructural characterization of HIV pseudovirions containing Gag and Env precursor proteins engineered in insect cells

AUTHOR(S): Tobin, Gregory J.; Nagashima, Kunio; Gonda, Matthew A.
CORPORATE SOURCE: Lab. Cell Molecular Structure, National Cancer Inst., Frederick, MD, 21702-1201, USA

SOURCE: Methods (San Diego) (1996), 10(2), 208-218
CODEN: MTHDE9; ISSN: 1046-2023

PUBLISHER: Academic

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Expression of human immunodeficiency virus (HIV) Gag precursor protein (Pr55) by recombinant baculoviruses in insect cells results in the **assembly** and budding of Pr55 as **virus-like particles**, or Gag pseudovirions. The ultrastructural morphol., size, and sucrose sedimentation rate of Gag pseudovirions are indistinguishable from immature lentivirus particles produced by HIV-infected human cells. Recombinant baculoviruses were engineered to express individually Pr55 and HIV Env glycoprotein precursor (gp160). These recombinant baculoviruses were used to co-infect insect cells to produce chimeric HIV Gag pseudovirions contg. gp160 in expts. to develop methodologies for producing complex noninfectious particulate vaccines for HIV. Coexpression of HIV Pr55 and gp160 resulted in the apparent **incorporation** of gp160 into Gag pseudovirions as detd. by immunoblotting with envelope-specific monoclonal antibodies. Furthermore, results from indirect immunogold electron microscopy using monoclonal antibodies to HIV gp120, a component of the Env glycoprotein precursor, suggested that HIV gp160 was specifically **incorporated** during the budding process into the outer surface of chimeric Gag pseudovirions. Parallel labeling expts. to localize gp120 and Pr55 epitopes on HIV-infected H9 lymphocytes provided results similar to those obtained

with chimeric Gag pseudovirions producing recombinant baculovirus-infected insect cells. Parameters influencing immunoelectron microscopy results in cell-surface and postembedding labeling expts. are discussed.

L51 ANSWER 37 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:699000 HCAPLUS

DOCUMENT NUMBER: 121:299000

TITLE: Localization of viral protein X in simian immunodeficiency virus macaque strain and analysis of its packaging requirements

AUTHOR(S): Liska, Vladimir; Spehner, Daniele; Mehtali, Majid; Schmitt, Doris; Kirn, Andre; Aubertin, Anne-Marie

CORPORATE SOURCE: Unite INSERM 74, Strasbourg, 67000, Fr.

SOURCE: Journal of General Virology (1994), 75(11), 2955-62
CODEN: JGVIAI; ISSN: 0022-1317

PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Simian immunodeficiency virus (SIV) and human immunodeficiency virus type 2 (HIV-2) encode the accessory viral protein X (Vpx) known to be **incorporated** into virions in amts. comparable to those of the Gag proteins. The localization of Vpx within SIVmac-infected HUT-78 cells and SIVmac virions was studied by immunoelectron microscopy. Vpx appeared to be assocd. with extracellular virions as well as budding viral particles at the surface of infected cells. Immunolabeling of purified viral cores suggested that Vpx was a component of the amorphous material surrounding the core structure. Furthermore, a detergent insol. fraction contg. SIV core proteins was devoid of Vpx. To investigate the protein requirement for packaging of Vpx, BHK-21 cells were co-infected with vaccinia virus recombinants encoding Vpx and other SIV proteins able to **assemble** into **virus-like particles**. Anal. by immunopptn. of the extracellular particulate material as well as immunoelectron microscopy demonstrated that co-expression of Vpx with the Pr56gag polyprotein was sufficient for the formation of pseudo-virions contg. Vpx. **Virus-like particles** that appeared upon expression of p16gag did not contain Vpx. The results suggest that Vpx is packaged into viral particles through its binding to the Gag polyprotein. The precise positioning of Vpx within the space sepg. the viral envelope from the core structure is postulated to result from the reorganization of viral proteins that occurs upon Gag polyprotein cleavage and budding.

L51 ANSWER 38 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:552739 HCAPLUS

DOCUMENT NUMBER: 121:152739

TITLE: Production and crystallization of **virus-like particles** assembled in a **heterologous protein** expression system

AUTHOR(S): McKinney, B. R.; Agrawal, D.; Fisher, A. J.; Johnson, J. E.; Schneemann, A.; Rueckert, R. R.

CORPORATE SOURCE: Dep. Biol. Sci., Purdue Univ., West Lafayette, IN, 47907, USA

SOURCE: Acta Crystallographica, Section D: Biological Crystallography (1994), D50(4), 351-4
CODEN: ABCRE6; ISSN: 0907-4449

DOCUMENT TYPE: Journal

LANGUAGE: English

AB It is of considerable interest to sep. the processes of viral infectivity

and virion assembly. Until recently this has only been possible with viruses that could be disassembled and reassembled in vitro. Even in these cases it was difficult to establish the authenticity of reassembled capsid protein because of possible irreversible damage that may have occurred to the protein during disassembly. An ideal method for the study of virus assembly is a protein expression system in which conditions are appropriate for spontaneous particle formation from freshly synthesized polypeptides. The baculovirus expression system has proven to be an excellent means to this end. Recently, this approach has been used to study the T = 3 Flock House insect virus and it has been demonstrated that subunits with the wild-type protein sequence, and with site-specific mutations that prevent particle maturation, will assemble and crystallize. This same approach has now been used at Purdue to study the T = 4 Nudaurelia .omega. capensis insect virus. There is no cell culture system currently available for the study of N.omega.V, thus the expression system provides the first opportunity to study assembly under controlled conditions.

L51 ANSWER 39 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:27253 HCAPLUS

DOCUMENT NUMBER: 120:27253

TITLE: Efficient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles

AUTHOR(S): Kirnbauer, Reinhard; Taub, Janet; Greenstone, Heather; Rogen, Richard; Duerst, Matthias; Gissmann, Lutz; Lowy, Doug R.; Schiller, John T.

CORPORATE SOURCE: Lab. Cell. Oncol., Natl. Cancer Inst., Bethesda, MD, 20892, USA

SOURCE: Journal of Virology (1993), 67(12), 6929-36
CODEN: JOVIAM; ISSN: 0022-538X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The L1 genes of 2 human papillomavirus type 16 (HPV16) isolates derived from condylomata acuminata were used to express the L1 major capsid protein in insect cells via recombinant baculoviruses. Both L1 major capsid proteins self-assembled into virus-like particles (VLP) with high efficiency and could be purified in preparative amts. on d. gradients. The yield of VLP was 3 orders of magnitude higher than what has been obtained previously, using L1 derived from the prototype HPV16. DNA sequence comparison identified a single nonconserved amino acid change to be responsible for the inefficient self-assembly of the prototype L1. VLP were also obtained by expressing L1 of HPV6, HPV11, and cottontail rabbit papillomavirus, indicating that L1 from a variety of papillomaviruses has the intrinsic capacity to self-assemble into VLP. Coexpression of HPV16 L1 plus L2 by using a baculovirus double-expression vector also resulted in efficient self-assembly of VLP, and the av. particle yield increased .apprx.4-fold in comparison to when L1 only was expressed. Coimmunopptn. of L1 and L2 and cosedimentation of the 2 proteins in a sucrose gradient demonstrated that L2 was incorporated into the particles. The ability to generate preparative amts. of HPV16 L1 and L1-L2 VLP may have implications for the development of a serol. assay to detect anti-HPV16 virion immune responses to conformational epitopes and for immunoprophylaxis against HPV16 infection.

L51 ANSWER 40 OF 52 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1992:35664 HCAPLUS

DOCUMENT NUMBER: 116:35664
 TITLE: Preparation of aggregates of an antigen using the self-assembling coat protein of a potyvirus
 INVENTOR(S): Jagadish, Mittur Nanjappa; Ward, Colin Wesley; Shukla, Dharma Deo
 PATENT ASSIGNEE(S): Commonwealth Scientific and Industrial Research Organization, Australia
 SOURCE: PCT Int. Appl., 47 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9115587	A1	19911017	WO 1991-AU128	19910405
W: AU, JP, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE				
AU 9176675	A1	19911030	AU 1991-76675	19910405
AU 639098	B2	19930715		
EP 527767	A1	19930224	EP 1991-907566	19910405
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
JP 05506145	T2	19930916	JP 1991-506696	19910405
PRIORITY APPLN. INFO.:				
			AU 1990-9508	19900406
			WO 1991-AU128	19910405

AB N- or C-terminal peptides of potyvirus coat protein can be replaced with foreign amino acid sequences without preventing coat protein folding and assembly into **virus-like particles**. The particles can be used in vaccines. Escherichia coli and Saccharomyces cerevisiae transformed with Johnson Grass Mosaic Virus coat protein cDNA produced coat protein that assembled into **virus-like particles**. E. coli producing a fusion protein in which the glutathione-S-transferase of Schistosoma japonicum replaced the N-terminal region of this coat protein also produced **virus-like particles**, although their morphol. differed from that of the native particles and there were fewer of them. The fusion proteins were immunogenic.

L51 ANSWER 41 OF 52 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1999:446873 BIOSIS
 DOCUMENT NUMBER: PREV199900446873
 TITLE: Expression of the alpha6 integrin confers papillomavirus binding upon receptor-negative B-cells.
 AUTHOR(S): McMillan, Nigel A.J. (1); Payne, Elizabeth; Frazer, Ian H.; Evander, Magnus
 CORPORATE SOURCE: (1) U.Q. Dept. of Medicine, PA Hospital, Ipswich Rd., Brisbane, QLD, 4102 Australia
 SOURCE: Virology, (Sept. 1, 1999) Vol. 261, No. 2, pp. 271-279. ISSN: 0042-6822.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 SUMMARY LANGUAGE: English

AB Papillomaviruses (PV) bind to a wide range of cell lines in a specific and saturable manner. We have recently identified a candidate receptor for papillomavirus as the alpha6 integrin (Evander et al., J. Virol. 71, 2449-2456, 1997). We have further investigated the role the alpha6 integrin plays in PV binding. Here we show that the cells expressing the alpha6 integrin, partnered with either the beta4 integrin or the beta1

integrin, are equally able to bind PV HPV6b L1 **virus-like particles**, indicating that the beta partner does not play a major role in virus binding. In order to provide definitive evidence that the alpha6 integrin is required for PV binding we undertook to genetically complement the receptor-negative B-cell line DG75 by expressing the human alpha6A gene. The transduction of the alpha6 integrin gene into DG75 cells results in the cell surface expression of the alpha6 protein and this expression confers upon DG75 cells the ability to bind laminin, a normal ligand for alpha6 integrin. Furthermore, the alpha6 protein is partnered with the beta1 integrin in DG75 cells. Finally, we show that the DG75-alpha6 cells were able to bind papillomavirus VLPs and this binding was inhibited by a functionally blocking anti-alpha6 antibody. Together these data indicate that the alpha6 integrin is a primary cell receptor for papillomaviruses and is both necessary and sufficient for PV binding.

L51 ANSWER 42 OF 52 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1992:264709 BIOSIS

DOCUMENT NUMBER: BA93:141034

TITLE: ROTAVIRUS VP3 EXPRESSED IN INSECT CELLS POSSESSES GUANYLYLTRANSFERASE ACTIVITY.

AUTHOR(S): LIU M; MATTION N M; ESTES M K

CORPORATE SOURCE: DIV. MOL. VIROL., BAYLOR COLLEGE MED., ONE BAYLOR PLAZA, HOUSTON, TEXAS 77030.

SOURCE: VIROLOGY, (1992) 188 (1), 77-84.

CODEN: VIRLAX. ISSN: 0042-6822.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB We have examined the possible function(s) of the protein VP3 encoded by the rotavirus SA11 genomic segment 3. Viral-associated VP3 in double-shelled and single-shelled particles was shown to bind GTP covalently and reversibly. These properties are similar to the unique characteristics of eukaryotic and viral guanylyltransferases, suggesting that VP3 is associated with a capping enzyme activity. Previous studies have shown that intact viral particles are required for transcription, making it difficult to unequivocally identify the functions of individual proteins within such particles. Characterization of VP3 produced in the baculovirus expression system showed that the expressed VP3 covalently bound GTP. These studies suggest that VP3 alone is the guanylyltransferase. GTP binding also was seen in core **virus-like particles** and single-shelled **virus-like particles** that lacked viral nucleic acid and were assembled in insect cells.

L51 ANSWER 43 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 2001:651263 SCISEARCH

THE GENUINE ARTICLE: 461LN

TITLE: Interaction of recombinant Norwalk virus particles with the 105-kilodalton cellular binding protein, a candidate receptor molecule for virus attachment

AUTHOR: Tamura M; Natori K; Kobayashi M; Miyamura T; Takeda N (Reprint)

CORPORATE SOURCE: Natl Inst Infect Dis, Dept Virol 2, Shinjuku Ku, 1-23-1 Toyama, Tokyo 1628640, Japan (Reprint); Natl Inst Infect Dis, Dept Virol 2, Shinjuku Ku, Tokyo 1628640, Japan; Univ Tokyo, Grad Sch Agr & Life Sci, Bunkyo Ku, Tokyo 1138657, Japan

COUNTRY OF AUTHOR: Japan

JOURNAL OF VIROLOGY, (DEC 2000) Vol. 74, No. 24, pp.

11589-11597.

Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW,
WASHINGTON, DC 20036-2904 USA.

ISSN: 0022-538X.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 69

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Norwalk virus (NV), responsible for outbreaks of acute gastroenteritis, comprises the species of the genus Norwalk-like viruses in the family Caliciviridae. Although the study of the molecular biology of NV has been hampered by a lack of culture systems or small experimental animal models, **virus-like particles** (VLPs) generated with recombinant baculoviruses harboring the capsid protein gene of NV provide a useful tool for investigating NV-cell interactions. In this study, the attachment of the recombinant VLPs derived from the Ueno virus (UEV), a strain belonging to the genogroup II NVs, to mammalian and insect cells was examined. Kinetic analyses of the binding of the recombinant VLPs of the UEV (rUEVs) to Caco-2 cells demonstrated that the binding was specific and occurred in a dose-dependent manner. Approximately 7.5% of the prebound rUEVs were internalized into the Caco-2 cells. Enzymatic and chemical modification of Caco-2 cell surface molecules suggested that the binding was directly mediated by a protein-protein interaction. A virus overlay protein-binding assay (VOPBA) indicated that rUEVs appeared to bind to a 105-kDa molecule, designated as the NV attachment (NORVA) protein. Furthermore, the assay indicated that its native conformational structure was indispensable for the binding activity. In Caco-2 cells, the NORVA protein was detected when VOPBA was carried out with the VLPs from Seto and Funabashi viruses, which are serologically different NVs from UEV, used as probes. The binding of rUEVs to NORVA protein was also observed in six mammalian cell lines other than Caco-2. These data suggest that the attachment of NV to mammalian cells is mediated by NORVA protein, which is ubiquitously expressed in the mammalian cells. The present study is the first report on the role of the cellular molecule in the binding of recombinant VLPs of NV.

L51 ANSWER 44 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 2001:20903 SCISEARCH

THE GENUINE ARTICLE: 384JB

TITLE: The B subunit of shiga toxin fused to a tumor antigen elicits CTL and targets dendritic cells to allow MHC class I-restricted presentation of peptides derived from exogenous antigens

AUTHOR: Haicheur N; Bismuth E; Bosset S; Adotevi O; Warnier G; Lacabanne V; Regnault A; Desaymard C; Amigorena S; Ricciardi-Castagnoli P; Goud B; Fridman W H; Johannes L; Tartour E (Reprint)

CORPORATE SOURCE: Univ Paris 06, Inst Curie, INSERM, U255, Unite Immunol Clin, F-75248 Paris 05, France (Reprint); Inst Curie, CNRS, UMR 144, Lab Mecanismes Mol Transport Intracellulaire, Paris, France; Inst Ludwig Canc Res, Brussels Branch, Brussels, Belgium; Inst Curie, INSERM, U520, Paris, France; Univ Milan, Bicocca, Dept Biosci & Biotechnol, Milan, Italy

COUNTRY OF AUTHOR: France; Belgium; Italy

SOURCE: JOURNAL OF IMMUNOLOGY, (15 SEP 2000) Vol. 165, No. 6, pp. 3301-3308.

Publisher: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE,
BETHESDA, MD 20814 USA.

ISSN: 0022-1767.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 63

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Immunization with peptide or recombinant proteins generally fails to elicit CTL, which are thought to play a key role in the control of virus-infected cells and tumor growth. In this study we show that the nontoxic B subunit of Shiga toxin fused to a tumor peptide derived from the mouse mastocytoma P815 can induce specific CTL in mice without the use of adjuvant. The Shiga B subunit acts as a vector rather than as an adjuvant, because coinjection of the tumor peptide and the B subunit as separate entities does not lead to CTL induction. We also demonstrated that in vitro the B subunit mediates the delivery of various exogenous CDS T cell epitopes into the conventional MHC class I-restricted pathway, as this process is inhibited by brefeldin A and lactacystin and requires a functional TAP system. In contrast to other nonviral methods for transport of exogenous Ags into the endogenous MHC class I pathway that involve macropinocytosis or phagocytosis, the Shiga B subunit targets this pathway in a receptor-dependent manner, namely via binding to the glycolipid Gb3. Because this **receptor** is highly **expressed** on various dendritic cells, it should allow preferential targeting of the Shiga B subunit to these professional APCs. Therefore, the Shiga B subunit appears to represent an attractive vector for vaccine development due to its ability to target dendritic cells and to induce specific CTL without the need for adjuvant.

L51 ANSWER 45 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1999:770922 SCISEARCH

THE GENUINE ARTICLE: 243AK

TITLE: The epithelial cell response to rotavirus infection

AUTHOR: Rollo E E; Kumar K P; Reich N C; Cohen J; Angel J; Greenberg H B; Sheth R; Anderson J; Oh B; Hempson S J; Mackow E R; Shaw R D (Reprint)

CORPORATE SOURCE: VET AFFAIRS MED CTR, DEPT MED, RES SERV, BLDG 62-151, NORTHPORT, NY 11768 (Reprint); VET AFFAIRS MED CTR, DEPT MED, RES SERV, NORTHPORT, NY 11768; SUNY STONY BROOK, DEPT PATHOL, STONY BROOK, NY 11794; INST NATL RECH ARGONOM, LAB VIROL & IMMUNOL MOL, JOUY EN JOSAS, FRANCE; STANFORD UNIV, STANFORD, CA 94305; PALO ALTO VET AFFAIRS MED CTR, DEPT MED, STANFORD, CA 94305; PALO ALTO VET AFFAIRS MED CTR, DEPT MICROBIOL, STANFORD, CA 94305

COUNTRY OF AUTHOR: USA; FRANCE

SOURCE: JOURNAL OF IMMUNOLOGY, (15 OCT 1999) Vol. 163, No. 8, pp. 4442-4452.

Publisher: AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.

ISSN: 0022-1767.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 79

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Rotavirus is the most important worldwide cause of severe gastroenteritis in infants and young children. Intestinal epithelial cells are the principal targets of rotavirus infection, but the response of enterocytes to rotavirus infection is largely unknown. We determined that rotavirus infection of HT-29 intestinal epithelial cells results in prompt activation of NF-kappa B (<2 h), STAT1, and ISG F3 (3 h). Genetically

inactivated rotavirus and **virus-like particles** assembled from baculovirus-expressed viral proteins also activated NF-kappa B. Rotavirus infection of HT-29 cells induced mRNA for several C-C and C-X-C chemokines as well as IFNs and GM-CSF. Mice infected with simian rotavirus or murine rotavirus responded similarly with the enhanced expression of a profile of C-C and C-X-C chemokines. The rotavirus-stimulated increase in chemokine mRNA was undiminished in mice lacking mast cells or lymphocytes. Rotavirus induced chemokines only in mice <15 days of age despite documented infection in older mice. Macrophage inflammatory protein-1 beta and IFN-stimulated protein 10 mRNA responses occurred, but were reduced in p50(-/-) mice. Macrophage inflammatory protein-1 beta expression during rotavirus infection localized to the intestinal epithelial cell in murine intestine. These results show that the intestinal epithelial cell is an active component of the host response to rotavirus infection.

L51 ANSWER 46 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)
 ACCESSION NUMBER: 1999:604624 SCISEARCH
 THE GENUINE ARTICLE: 221MB
 TITLE: Genetically controlled cell lysis in the yeast
 Saccharomyces cerevisiae
 AUTHOR: Zhang N S; Gardner D C J; Oliver S G; Stateva L I
 (Reprint)
 CORPORATE SOURCE: UMIST, DEPT BIOMOL SCI, POB 88, MANCHESTER M60 1QD, LANCS,
 ENGLAND (Reprint); UMIST, DEPT BIOMOL SCI, MANCHESTER M60
 1QD, LANCS, ENGLAND
 COUNTRY OF AUTHOR: ENGLAND
 SOURCE: BIOTECHNOLOGY AND BIOENGINEERING, (5 SEP 1999) Vol. 64,
 No. 5, pp. 607-615.
 Publisher: JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK,
 NY 10158-0012.
 ISSN: 0006-3592.
 DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: LIFE; AGRI
 LANGUAGE: English
 REFERENCE COUNT: 42

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The cell wall of the yeast *Saccharomyces cerevisiae* is a tough, rigid structure, which presents a significant barrier to the release of native or recombinant proteins from this biotechnologically important organism. There is hence a need to develop inexpensive and efficient methods of lysing yeast cells in order to release their intracellular contents. To develop such a method, a tightly regulated promoter, pMET3, has been used to control three genes involved in cell wall biogenesis: PDE2, SRB1/PSA1, and PKC1. Two of these regulation cassettes, pMET3-SRB1/PSA1 and pMET3-PKC1, have been integrated at the chromosomal loci of the respective genes in order to overcome problems of plasmid instability. Although repression of PDE2 did not cause cell lysis, cells depleted of *Srb1p/Psalp* gradually lost their viability and integrity, releasing about 10% of total protein into the medium. Repression of PKC1 led to extensive cell lysis, accompanied by the release of 45% of cellular protein into the medium. A double mutant, carrying both pMET3-SRB1/PSA1 and pMET3-PKC1 cassettes in place of SRB1/PSA1 and PKC1, was constructed and found to permit the efficient release of both homologous and **heterologous proteins**. (C) 1999 John Wiley & Sons, Inc.

L51 ANSWER 47 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)
 ACCESSION NUMBER: 1999:544893 SCISEARCH
 THE GENUINE ARTICLE: 213ZV

TITLE: Efficient formation of influenza **virus-like particles**: dependence on the expression levels of viral proteins
AUTHOR: GomezPuertas P; Mena I; Castillo M; Vivo A; PerezPastrana E; Portela A (Reprint)
CORPORATE SOURCE: INST SALUD CARLOS III, CTR NACL BIOL FUNDAMENTAL, MADRID 28220, SPAIN (Reprint); INST SALUD CARLOS III, CTR NACL BIOL FUNDAMENTAL, MADRID 28220, SPAIN; INST SALUD CARLOS III, CTR NACL MICROBIOL, MADRID 28220, SPAIN
COUNTRY OF AUTHOR: SPAIN
SOURCE: JOURNAL OF GENERAL VIROLOGY, (JUL 1999) Vol. 80, Part 7, pp. 1635-1645.
Publisher: SOC GENERAL MICROBIOLOGY, MARLBOROUGH HOUSE, BASINGSTOKE RD, SPENCERS WOODS, READING RG7 1AE, BERKS, ENGLAND.
ISSN: 0022-1317.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 50

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB It has previously been demonstrated in this laboratory that an influenza virus-like chloramphenicol acetyltransferase (CAT) RNA could be expressed in COS-1 cells that synthesized all ten influenza A virus-encoded proteins from recombinant plasmids. It was also shown that supernatant fluids harvested from these cultures contained **virus-like particles** (VLPs) that could deliver an enclosed CAT RNA to MDCK cells. Here, it is shown that the levels of expression of the reporter gene in the COS-1 and/or MDCK cells can be altered drastically by modifying the concentrations of the recombinant plasmids transfected in the COS-1 cells. Thus, it was observed that overexpression of NS2 reduced CAT expression in COS-1 cells, whereas overexpression of M2 and NS1 proteins dramatically decreased transmission of the CAT RNA to the MDCK cultures. These results are discussed with reference to the roles of these proteins during virus replication. From these experiments, a ratio of transfected plasmids was found that increased the efficiency of the previously described system by 50-100-fold. Under these optimized conditions, it was demonstrated that VLPs can be formed in the absence of neuraminidase expression and that these VLPs remained aggregated to each other and to cell membranes. Moreover, it is shown that CAT RNA transmission was dependent on specific interactions of the ribonucleoprotein complex with other viral structural polypeptides. These data demonstrate the usefulness of this encapsidation-packaging system for the study of different aspects of the influenza virus life-cycle.

L51 ANSWER 48 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1998:77294 SCISEARCH

THE GENUINE ARTICLE: YQ942

TITLE: Mutational analysis of the human immunodeficiency **virus** type 1 Vpu transmembrane domain that promotes the enhanced release of **virus-like particles** from the plasma membrane of mammalian cells

AUTHOR: Paul M; Mazumder S; Raja N; Jabbar M A (Reprint)

CORPORATE SOURCE: EMORY UNIV, SCH MED, EMORY VACCINE RES CTR, DEPT IMMUNOL & MICROBIOL, G 211 ROLLINS RES BLDG, ATLANTA, GA 30322 (Reprint); CLEVELAND CLIN FDN, DEPT MOL BIOL, LERNER RES INST, CLEVELAND, OH 44195

COUNTRY OF AUTHOR: USA

SOURCE: JOURNAL OF VIROLOGY, (FEB 1998) Vol. 72, No. 2, pp. 1270-1279.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 73

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Human immunodeficiency **virus** type 1 Vpu is a multifunctional phosphoprotein composed of the N-terminal transmembrane (VpuTM) and C-terminal cytoplasmic domains. Each of these domains regulates a distinct function of the protein; the transmembrane domain is critical in **virus** release, and phosphorylation of the cytoplasmic domain is necessary for CD4 proteolysis. We carried out experiments to identify amino acids in the VpuTM domain that are important in the process of **virus-like particle (VLP)** release from HeLa cells. VLPs are released from the plasma membrane of HeLa cells at constitutive levels, and Vpu expression enhanced the release of VLPs by a factor of 10 to 15. Deletion of two to five amino acids from both N- and C-terminal ends or the middle of the VpuTM domain generated mutual Vpu proteins that have lost the ability to enhance **VLP** release. These deletion mutants have not lost the ability to associate with the wild-type or mutual Vpu proteins and formed complexes with equal efficiency. They were also transported normally to the Golgi complex. Furthermore, a Vpu protein having the CD4 transmembrane and Vpu cytoplasmic domains was completely inactive, and Vpu proteins harboring hybrid Vpu-CD4 TM domains were also defective in the ability to enhance the release of VLPs. When tested for functional complementation in cotransfected cells, two inactive proteins were not able to reconstitute Vpu activity that enhances the release of Gag particles. Coexpression of functional CD4/Vpu hybrids or wild-type Vpu with inactive mutant CD4/Vpu proteins revealed that mutations in the VpuTM domain could dominantly interfere with Vpu activity in the Gag release. Taken together, these results demonstrated that the structural integrity of the VpuTM domain is critical for Vpu activity in the release of VLPs from the plasma membrane of mammalian cells.

L51 ANSWER 49 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1998:57647 SCISEARCH

THE GENUINE ARTICLE: YQ046

TITLE: Recovery of homogeneous and functional beta(2)-adrenergic receptors from extracellular baculovirus particles

AUTHOR: Loisel T P; Ansanay H; StOnge S; Gay B; Boulanger P; Strosberg A D; Marullo S; Bouvier M (Reprint)

CORPORATE SOURCE: UNIV MONTREAL, DEPT BIOCHIM, MONTREAL, PQ H3C 3J7, CANADA (Reprint); UNIV MONTREAL, DEPT BIOCHIM, MONTREAL, PQ H3C 3J7, CANADA; UNIV MONTREAL, GRP RECH SYST NERVEUX AUTONOME, MONTREAL, PQ H3C 3J7, CANADA; FAC MED MONTPELLIER, INST BIOL, LAB VIROL & PATHOGENESES MOL, CNRS, URA 1487, F-34060 MONTPELLIER, FRANCE; CNRS, INST COCHIN GENET MOL, UPR 415, LAB IMMUNOPHARMACOL MOL, F-75014 PARIS, FRANCE; UNIV PARIS 07, F-75014 PARIS, FRANCE

COUNTRY OF AUTHOR: CANADA; FRANCE

SOURCE: NATURE BIOTECHNOLOGY, (NOV 1997) Vol. 15, No. 12, pp. 1300-1304.

Publisher: NATURE PUBLISHING CO, 345 PARK AVE SOUTH, NEW

YORK, NY 10010-1707.
ISSN: 1087-0156.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 39

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Expression in baculovirus-infected insect cells allows sufficient production of **G-protein coupled receptor** for structural studies. An important drawback of this expression system comes from the presence of unprocessed and biologically inactive receptors that have to be eliminated during receptor purification steps. We show that viral particles released from Sf9 cells infected with a recombinant baculovirus coding for the human beta(2)-adrenergic receptor (beta(2)AR) cDNA contain glycosylated and biologically active beta(2)AR. In addition, post-translational modifications known to modulate receptor activity were found to occur in these particles.

L51 ANSWER 50 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 97:823773 SCISEARCH

THE GENUINE ARTICLE: YD986

TITLE: Recovery of homogeneous and functional beta(2)-adrenergic receptors from extracellular baculovirus particles

AUTHOR: Loisel T P; Ansanay H; StOnge S; Gay B; Boulanger P; Strosberg A D; Marullo S; Bouvier M (Reprint)

CORPORATE SOURCE: UNIV MONTREAL, DEPT BIOCHIM, MONTREAL, PQ H3C 3J7, CANADA (Reprint); UNIV MONTREAL, DEPT BIOCHIM, MONTREAL, PQ H3C 3J7, CANADA; UNIV MONTREAL, GRP RECH SYST NERVEUX AUTONOME, MONTREAL, PQ H3C 3J7, CANADA; FAC MED MONTELLIER, INST BIOL, LAB VIROL & PATHOGENESES MOL, CNRS, URA 1487, F-34060 MONTELLIER, FRANCE; INST COCHIN GENET MOL, LAB IMMUNOPHARMACOL MOL, CNR, UPR 415, F-75014 PARIS, FRANCE; UNIV PARIS 07, F-75014 PARIS, FRANCE

COUNTRY OF AUTHOR: CANADA; FRANCE

SOURCE: NATURE BIOTECHNOLOGY, (NOV 1997) Vol. 15, No. 11, pp. 1300-1304.

Publisher: NATURE PUBLISHING CO, 345 PARK AVE SOUTH, NEW YORK, NY 10010-1707.

ISSN: 1087-0156.

DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: English
REFERENCE COUNT: 39

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Expression in baculovirus-infected insect cells allows sufficient production of **G-protein coupled receptor** for structural studies. An important drawback of this expression system comes from the presence of unprocessed and biologically inactive receptors that have to be eliminated during receptor purification steps. We show that viral particles released from Sf9 cells infected with a recombinant baculovirus coding for the human beta(2)-adrenergic receptor (beta(2)AR) cDNA contain glycosylated and biologically active beta(2)AR. In addition, post-translational modifications known to modulate receptor activity were found to occur in these particles.

L51 ANSWER 51 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 97:38046 SCISEARCH

THE GENUINE ARTICLE: WA167

TITLE: Characterization of the expression and immunogenicity of

poliovirus replicons that encode simian immunodeficiency virus SIV(mac)239 gag or envelope SU proteins

AUTHOR: Anderson M J; Porter D C; Moldoveanu Z; Fletcher T M; McPherson S; Morrow C D (Reprint)

CORPORATE SOURCE: UNIV ALABAMA, DEPT MICROBIOL, 619 LYONS HARRISON RES BLDG, 1900 7TH AVE S, BIRMINGHAM, AL 35294 (Reprint); UNIV ALABAMA, DEPT MICROBIOL, BIRMINGHAM, AL 35294; UNIV ALABAMA, DEPT MED, BIRMINGHAM, AL 35294; UNIV ALABAMA, CTR AIDS RES, BIRMINGHAM, AL 35294

COUNTRY OF AUTHOR: USA

SOURCE: AIDS RESEARCH AND HUMAN RETROVIRUSES, (1 JAN 1997) Vol. 13, No. 1, pp. 53-62.
 Publisher: MARY ANN LIEBERT INC PUBL, 2 MADISON AVENUE, LARCHMONT, NY 10538.
 ISSN: 0889-2229.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 36

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The effectiveness of the poliovirus vaccines to induce both systemic and mucosal immunity has prompted the development of this virus as a vector in which to express foreign proteins. Our laboratory has previously reported on the construction and characterization of poliovirus genomes that encode HIV-1 proteins (Porter DC, et al.: 3 Virol 1996;70:2643-2649). To develop this system further, we have constructed poliovirus genomes, referred to as replicons, which encode the SIV(mac)239 Gag or Env SU in place of the poliovirus capsid gene (Pi). Since the replicons do not encode capsid proteins, they are encapsidated into poliovirions by passage with a recombinant vaccinia virus, WPl, which provides the poliovirus capsid proteins in trans. Using this system, we have derived stocks of the encapsidated replicons which encode the SIV(mac)239 Gag or Env SU protein. Infection of cells with the replicon that encodes SIV(mac)239 Gag resulted in the expression of a 55-kDa protein that was released from the infected cells. Analysis of the sedimentation of the released proteins by sucrose density gradient centrifugation revealed that the protein was released from the cell in the form of a **virus-like particle**. Infection of cells with the replicons encoding the SIV(mac)239 Env SU resulted in the expression of a 63-kDa protein, corresponding to the molecular mass predicted for the nonglycosylated SIV(mac)239 SU protein. A second protein with a molecular mass greater than 160 kDa was also immunoprecipitated. After enzymatic deglycosylation, this protein migrated at a molecular mass consistent with that for an Env SU dimer. Analysis of the medium from cells infected with the replicon encoding SIV(mac)239 Env SU revealed the presence of a protein of molecular mass 85-90 kDa, possibly representing a fragment of the SIV(mac)239 Env SU protein. To determine the immunogenicity of the replicons encoding SIV(mac)239 Gag or Env SU, transgenic mice that **express** the human **receptor** for poliovirus, and are thus susceptible to poliovirus, were immunized via the intramuscular route. A serum antibody response to SIV envelope was detected following booster immunization, establishing that the encapsidated replicon was immunogenic. Finally, we demonstrate that the replicons have the capacity to infect peripheral blood mononuclear monocytes/macrophages, suggesting that this cell is a possible target for in vivo infection. The results of our studies, then, lend further support for the development and application of recombinant poliovirus replicons in a vaccine strategy.

L51 ANSWER 52 OF 52 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 94:514711 SCISEARCH
THE GENUINE ARTICLE: PB785
TITLE: EFFICIENCY AND SELECTIVITY OF RNA PACKAGING BY
ROUS-SARCOMA VIRUS GAG DELETION MUTANTS
AUTHOR: SAKALIAN M; WILLS J W; VOGT V M (Reprint)
CORPORATE SOURCE: CORNELL UNIV, BIOCHEM MOLEC & CELL BIOL SECT, BIOTECHNOL
BLDG, ITHACA, NY, 14853 (Reprint); CORNELL UNIV, BIOCHEM
MOLEC & CELL BIOL SECT, ITHACA, NY, 14853; PENN STATE
UNIV, MILTON S HERSHEY MED CTR, DEPT MICROBIOL & IMMUNOL,
HERSHEY, PA, 17033
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF VIROLOGY, (SEP 1994) Vol. 68, No. 9, pp.
5969-5981.
ISSN: 0022-538X.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 59

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In all retrovirus systems studied, the leader region of the RNA contains a cis-acting sequence called Psi that is required for packaging the viral RNA genome. Since the pol and env genes are dispensable for formation of RNA-containing particles, the **gag gene** product must have an RNA binding domain(s) capable of recognizing Psi. To gain information about which portion(s) of Gag is required for RNA packaging in the avian sarcoma and leukemia virus system, we utilized a series of gag deletion mutants that retain the ability to **assemble virus-like particles**. COS cells were cotransfected with these mutant DNAs plus a tester DNA containing Psi, and **incorporation** of RNA into particles was measured by RNase protection. The efficiency of packaging was determined by normalization of the amount of Psi(+) RNA to the amount of Gag protein released in **virus-like particles**. Specificity of packaging was determined by comparisons of Psi(+) and Psi(-) RNA in particles and in cells. The results indicate that much of the MA domain, much of the p10 domain, half of the CA domain, and the entire PR domain of Gag are unnecessary for efficient packaging. In addition, none of these deleted regions is needed for specific selection of the Psi RNA. Deletions within the NC domain, as expected, reduce or eliminate both the efficiency and the specificity of packaging. Among mutants that retain the ability to package, a deletion within the CA domain (which includes the major homology region) is the least efficient. We also examined particles of the well-known packaging mutant SE21Q1b. The data suggest that the random RNA packaging behavior of this mutant is not due to a specific defect but rather is the result of the cumulative effect of many point mutations throughout the **gag gene**.